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(54) Title: IDENTIFICATION OF BROADLY REACTIVE DR RESTRICTED EPITOPES

(57) Abstract

The present invention is based, at least in part, on the discovery and validation of specific immunogenic peptide epitopes for various HLA class II DR molecules, representative of the worldwide population. Such peptides comprise an epitope, or analog thereof, which binds to an HLA class II molecule at an IC_{50} of less than or equal to 1,000 nM.

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IDENTIFICATION OF BROADLY REACTIVE DR RESTRICTED EPITOPES

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of Provisional U.S.S.N. 60/087,192 filed 5/29/98. The application is also related to U.S.S.N. 09/009953, filed January 21, 1998, U.S.S.N. 60/036,713, filed January 23, 1997, and U.S.S.N. 60/037,432 filed February 7, 1997.

BACKGROUND OF THE INVENTION

Helper T lymphocytes (HTL) play several important functions in immunity to pathogens. Firstly, they provide help for induction of both CTL and antibody responses. By both direct contact and by secreting lymphokines such as IL2 and IL4, HTL promote and support the expansion and differentiation of T and B cell precursors into effector cells. In addition, HTL can also be effectors in their own right, an activity also mediated by direct cell contact and secretion of lymphokines, such as IFNγ and TNFα. HTL have been shown to have direct effector activity in case of tumors, as well as viral, bacterial, parasitic, and fungal infections.

HTL recognize a complex formed between class II MHC molecules and antigenic peptides, usually between 10 and 20 residues long, and with an average size of between 13 and 16 amino acids. Peptide-class II interactions have been analyzed in detail, both at the structural and functional level, and peptide motifs specific for various human and mouse class II molecules have been proposed.

In the last few years, epitope based vaccines have received considerable attention as a possible mean to develop novel prophylactic vaccines and immunotherapeutic strategies. Selection of appropriate T and B cell epitopes should allow to focus the immune system toward conserved epitopes of pathogens which are characterized by high sequence variability (such as HIV, HCV and Malaria).

In addition, focusing the immune response towards selected determinants could be of value in the case of various chronic viral diseases and cancer, where T cells directed against the immunodominant epitopes might have been inactivated while T cells specific for subdominant epitopes might have escaped T cell tolerance. The use of epitope

based vaccines also allows to avoid "suppressive" T cell determinants which induce TH_2 responses, in conditions where a TH_1 response is desirable, or vice versa.

Finally, epitope based vaccines also offer the opportunity to include in the vaccine construct epitopes that have been engineered to modulate their potency, either by increasing MHC binding affinity, or by alteration of its TCR contact residues, or both. Inclusion of completely synthetic non-natural or generically unrelated to the pathogen epitopes (such as TT derived "universal" epitopes), also represents a possible mean of modulating the HTL response toward a TH_1, or TH_2 phenotype.

Once appropriate epitope determinants have been defined, they can be assorted and delivered by various means, which include lipopeptides, viral delivery vectors, particles of viral or synthetic origin, naked or particle absorbed cDNA.

However, before appropriate epitopes can be defined, one major obstacle has to be overcome, namely the very high degree of polymorphism of the MHC molecules expressed in the human population. In fact, more than two hundred different types of HLA class I and class II molecules have already been identified. It has been demonstrated that in the case of HLA class I molecules, peptides capable of binding several different HLA class I molecules can be identified. Over 60% of the known HLA class I molecules can, in fact, be grouped in four broad HLA supertypes, characterized by similar peptide binding specificities (HLA supermotifs).

In the case of class II molecules, it is also known that peptides capable of binding multiple HLA types and of being immunogenic in the context of different HLA molecules do indeed exist. Specific immunogenic peptide have not been readily identified, particularly those reaactive with a large number of allelic products.

The present invention addresses these and other needs.

SUMMARY OF THE INVENTION

The present invention is based, at least in part, on the discovery and validation of specific immunogenic peptide epitopes for various HLA class II DR molecules, representative of the worldwide population. Such peptides comprise an epitope, or analog thereof, which binds to an HLA class II molecule at an IC_{50} of less than or equal to 1,000 nM. Epitopes of the invention have been identified in a variety of antigens including tumor associated antigens such as carcinoembryonic antigen (CEA), p53, MAGE-2, MAGE-3, or

Her2/neu; viral antigens such as those from HIV, HBV, or HCV; and parasites such as *Plasmodium falciparum*.

The HLA class II binding peptides of the invention may further comprise an epitope having an amino acid that is Y, F, W, L, I, V, or M at the first position from the N-terminus of the epitope and an amino acid of S, T, C, A, P, V, I, L, or M at the sixth position from the N-terminus of the epitope.

A peptide epitope of the invention, or a nucelic acid that encodes a peptide of the invention, may be used, *inter alia*, as a pharmaceutical composition to induce a helper T cell response in a patient by contacting a helper T cell with the epitope. One or more peptide epitopes of the invention may be included in such a composition. In a preferred embodiment, one or more epitopes is presented to a helper T cell by an antigen-presenting cell that has been pulsed with the peptide *ex vivo*.

Definitions

The term "peptide" is used interchangeably with "oligopeptide" in the present specification to designate a series of residues, typically L-amino acids, connected one to the other typically by peptide bonds between the alpha-amino and carbonyl groups of adjacent amino acids. The oligopeptides of the invention are typically less than about 50 residues in length and usually consist of between about 10 and about 30 residues, more usually between about 12 and 25, and often 15 and about 20 residues.

An "immunogenic peptide" is a peptide which comprises an allele-specific motif such that the peptide will bind an MHC molecule and induce an HTL response. Immunogenic peptides of the invention are capable of binding to an appropriate HLA molecule and inducing HTL response against the antigen from which the immunogenic peptide is derived.

With regard to a particular amino acid sequence, an "epitope" is a set of amino acid residues which is involved in recognition by a particular immunoglobulin, or in the context of T cells, those residues necessary for recognition by T cell receptor proteins and/or Major Histocompatibility Complex (MHC) receptors. In an immune system setting, *in vivo* or *in vitro*, an epitope is the collective features of a molecule, such as primary, secondary and tertiary peptide structure, and charge, that together form a site recognized by an immunoglobulin, T cell receptor or HLA molecule.

A "conserved residue" is a conserved amino acid occupying a particular position in a peptide motif typically one where the MHC structure may provide a contact point with the immunogenic peptide. One to three, typically two, conserved residues within a peptide of defined length defines a motif for an immunogenic peptide. These residues are typically in close contact with the peptide binding groove, with their side chains buried in specific pockets of the groove itself.

The term "motif" refers to the pattern of residues in a peptide of defined length, usually a peptide of from about 8 to about 13 amino acids for a class I HLA motif and from about 6 to about 25 amino acids for a class II HLA motif, which is recognized by a particular HLA molecule. Peptide motifs are typically different for each protein encoded by each human HLA allele and differ in the pattern of the primary and secondary anchor residues.

The term "supermotif" refers to motifs that, when present in an immunogenic peptide, allow the peptide to bind more than one HLA antigen. The supermotif preferably is recognized by at least one HLA allele having a wide distribution in the human population, preferably recognized by at least two alleles, more preferably recognized by at least three alleles, and most preferably recognized by more than three alleles.

A "primary anchor residue" is an amino acid at a specific position along a peptide sequence which is understood to provide a contact point between the immunogenic peptide and the HLA molecule. One to three primary anchor residues within a peptide of defined length generally defines a "motif" for an immunogenic peptide. These residues are understood to fit in close contact with peptide binding grooves of an HLA molecule, with their side chains buried in specific pockets of the binding grooves themselves. For example, analog peptides can be created by altering the presence or absence of particular residues in these primary anchor positions. Such analogs are used to modulate the binding affinity of a peptide comprising a particular motif or supermotif.

A "secondary anchor residue" is an amino acid at a position other than a primary anchor position in a peptide which may influence peptide binding. A secondary anchor residue occurs at a significantly higher frequency amongst bound peptides than would be expected by random distribution of amino acids at one position. The secondary anchor residues are said to occur at "secondary anchor positions." A secondary anchor residue can be identified as a residue which is present at a higher frequency among high or intermediate affinity binding peptides, or a residue otherwise associated with high or intermediate affinity

binding. For example, analog peptides can be created by altering the presence or absence of particular residues in these secondary anchor positions. Such analogs are used to finely modulate the binding affinity of a peptide comprising a particular motif or supermotif.

A "negative binding residue" is an amino acid which if present at certain positions (typically not primary anchor positions) of peptide epitope results in decreased binding affinity of the peptide for the peptide's corresponding HLA molecule.

Throughout this disclosure, results are expressed in terms of "IC_{50}'s." IC_{50} is the concentration of peptide in a binding assay at which 50% inhibition of binding of a reference peptide is observed. Given the conditions in which the assays are run (*i.e.*, limiting HLA proteins and labeled peptide concentrations), these values approximate K_D values. It should be noted that IC_{50} values can change, often dramatically, if the assay conditions are varied, and depending on the particular reagents used (*e.g.*, HLA preparation, *etc.*). For example, excessive concentrations of HLA molecules will increase the apparent measured IC_{50} of a given ligand. Assays for determining binding are described in detail in PCT publications WO 94/20127 and WO 94/03205. Alternatively, binding is expressed relative to a reference peptide. As a particular assay becomes more, or less, sensitive, the IC_{50}'s of the peptides tested may change somewhat. However, the binding relative to the reference peptide will not significantly change. For example, in an assay run under conditions such that the IC_{50} of the reference peptide increases 10-fold, the IC_{50} values of the test peptides will also shift approximately 10-fold. Therefore, to avoid ambiguities, the assessment of whether a peptide is a good, intermediate, weak, or negative binder is generally based on its IC_{50}, relative to the IC_{50} of a standard peptide.

As used herein, "high affinity" with respect to HLA class II molecules is defined as binding with an IC_{50} or K_D of less than 100 nM. "Intermediate affinity" is binding with an IC_{50} or K_D of between about 100 and about 1000 nM.

"Cross-reactive binding" indicates that a peptide is bound by more than one HLA molecule; a synonym is degenerate binding.

The phrases "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany it as found in its native state. Thus, the peptides of this invention do not contain materials normally associated with their *in situ* environment, e.g., MHC I molecules on antigen presenting cells. Even where a protein has been isolated to a homogenous or dominant band, there are trace contaminants in the range of 5-10% of native protein which co-purify with the desired

protein. Isolated peptides of this invention do not contain such endogenous co-purified protein.

The term "residue" refers to an amino acid or amino acid mimetic incorporated in an oligopeptide by an amide bond or amide bond mimetic.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 presents a map of the positive or negative effect of each of the 20 naturally occurring amino acids on DR4w4 binding capacity when occupying a particular position, relative to the main P1-P6 anchors.

Figure 2A presents a map of the positive or negative effect of each of the 20 naturally occurring amino acids on DR1 binding capacity when occupying a particular position, relative to the main P1-P6 anchors.

Figure 2B presents a map of the positive or negative effect of each of the 20 naturally occurring amino acids on DR7 binding capacity when occupying a particular position, relative to the main P1-P6 anchors.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention relates to compositions and methods for preventing, treating or diagnosing a number of pathological states such as viral, fungal, bacterial and parasitic diseases and cancers. In particular, it provides novel peptides capable of binding selected major histocompatibility complex (MHC) class II molecules at an IC_{50} of less than or equal to 1000 nM and inducing an immune response.

Peptide binding to MHC molecules is determined by the allelic type of the MHC molecule and the amino acid sequence of the peptide. MHC class II-binding peptides usually contain within their sequence two conserved ("anchor") residues that interact with corresponding binding pockets in the MHC molecule. Specific combination of anchor residues (usually referred to as "MHC motifs") required for binding by several allelic forms of human MHC (HLA, histocompatibility leukocyte antigens) are described in International Applications WO 94/03205 and WO 94/20127. Definition of specific MHC motifs allows one to predict from the amino acid sequence of an individual protein, which peptides have the potential of being immunogenic for HTL. These applications describe methods for preparation and use of immunogenic peptides in the treatment of disease.

An affinity threshold strongly correlated with immunogenicity in the context of HLA class II DR molecules has been delineated as disclosed herein. In order to define a biologically significant threshold of DR binding affinity, a database of the binding affinities of 32 DR-restricted epitopes for their restricting element (*i.e.*, the HLA molecule that binds the motif) was compiled. In approximately half of the cases (15 of 32 epitopes), DR restriction was associated with high binding affinities, i.e. binding affinities of less than 100 nM. In the other half of the cases (16 of 32), DR restriction was associated with intermediate affinity (binding affinities in the 100-1000 nM range). In only one of 32 cases was DR restriction associated with an IC_{50} of 1000 nM or greater. Thus, 1000 nM, preferably 100 nM, can be defined as an affinity threshold associated with immunogenicity in the context of DR molecules.

The peptide epitopes described here can also be used in combination with peptide epitopes which induce a CTL response. See, also, WO 95/07077.

The peptide epitopes of the invention may also include analogs of the epitopes. Although the peptide epitopes may exhibit cross-reactive binding with multiple DR alleles, cross-reactivity is not always complete and in such cases procedures to further increase cross-reactivity of peptides can be useful; such procedures can also be used to modify other properties of the peptide epitopes. Having established the general rules that govern cross-reactivity of peptides for HLA alleles within a given motif or supermotif, modification (*i.e.*, analoging) of the structure of peptides of particular interest in order to achieve broader (or otherwise modified) HLA binding capacity can be performed. More specifically, peptides which exhibit the broadest cross-reactivity patterns, (both amongst the known T cell epitopes, as well as the more extended set of peptides that contain the appropriate supermotifs), can be produced in accordance with the teachings herein.

The strategy employed utilizes the motifs or supermotifs which correlate with binding to certain HLA molecules. The motifs or supermotifs are defined by having primary anchors, though secondary anchors can also be modified. Analog peptides can be created by substituting amino acids residues at primary anchor, secondary anchor, or at primary and secondary anchor positions. Generally, analogs are made for peptides that already bear a motif or supermotif. Preferred secondary anchor residues of supermotifs and motifs that have been defined for HLA class II binding peptides are shown in Table IX.

For a number of the motifs or supermotifs in accordance with the invention, residues are defined which are deleterious to binding to allele-specific HLA molecules or

members of HLA supertypes that bind to the respective motif or supermotif. Accordingly, removal of residues that are detrimental to binding can be performed in accordance with the present invention. For example, in the case of the A3 supertype, when all peptides that have such deleterious residues are removed from the population of analyzed peptides, the incidence of cross-reactivity increases from 22% to 37% (see, e.g., Sidney, J. *et al.*, *Hu. Immunol.* 45:79, 1996). Thus, one strategy to improve the cross-reactivity of peptides within a given supermotif is simply to delete one or more of the deleterious residues present within a peptide and substitute a small "neutral" residue such as Ala (that may not influence T cell recognition of the peptide). An enhanced likelihood of cross-reactivity is expected if, together with elimination of detrimental residues within a peptide, residues associated with high affinity binding to multiple alleles within a superfamily are inserted.

To ensure that changes in the native or original epitope recognized by T cells do not lead to a failure to elicit helper T cells that cross-react with the wild type peptides, the variant peptide may be used to immunize T cells *in vitro* from individuals of the appropriate HLA allele, and the cells' capacity to induce lysis of wild type peptide sensitized target cells is evaluated. In both class I and class II systems it will be desirable to use as targets, cells that have been either infected or transfected with the appropriate genes to establish whether endogenously produced antigen is also recognized by the relevant T cells.

Another embodiment of the invention to ensure adequate numbers of cross-reactive cellular binders is to create analogs of weak binding peptides. Class II peptides exhibiting binding affinities of above 1000 nM, and carrying an acceptable but suboptimal primary anchor residue at one or both positions can be "fixed" by substituting preferred anchor residues in accordance with the respective supertype. The analog peptides can then be tested for crossbinding activity.

Another embodiment for generating effective peptide analogs involves the substitution of residues that have an adverse impact on peptide stability or solubility in a liquid environment. This substitution may occur at any position of the peptide epitope. For example, a cysteine (C) can be substituted out in favor of α-amino butyric acid. Due to its chemical nature, cysteine has the propensity to form disulfide bridges and sufficiently alter the peptide structurally so as to reduce binding capacity. Substituting α-amino butyric acid for C not only alleviates this problem, but actually improves binding and crossbinding capability in certain instances (Review: A. Sette et al, In: Persistent Viral Infections, Eds. R. Ahmed and I. Chen, John Wiley & Sons, England, in press, 1998). Substitution of cysteine

with α-amino butyric acid may occur at any residue of a peptide epitope, i.e. at either anchor or non-anchor positions.

The DR-binding peptides of the present invention or nucleic acids encoding them can be administered to mammals, particularly humans, for prophylactic and/or therapeutic purposes. The DR peptide epitopes can be used to enhance immune responses against other immunogens administered with the peptides. For instance, CTLepitope/DR epitope mixtures may be used to treat and/or prevent viral infection and cancer. Alternatively, immunogens which induce antibody responses can be used. Examples of diseases which can be treated using the immunogenic mixtures of DR peptides and other immunogens include prostate cancer, hepatitis B, hepatitis C, AIDS, renal carcinoma, cervical carcinoma, lymphoma, CMV and condyloma acuminatum.

The DR-binding peptides or nucleic acids encoding them may also be used to treat a variety of conditions involving unwanted T cell reactivity. Examples of diseases which can be treated using DR-binding peptides include autoimmune diseases (e.g., rheumatoid arthritis, multiple sclerosis, and myasthenia gravis), allograft rejection, allergies (e.g., pollen allergies), lyme disease, hepatitis, LCMV, post-streptococcal endocarditis, or glomerulonephritis, and food hypersensitivities.

In therapeutic applications, the immunogenic compositions or the DR-binding peptides or nucleic acids of the invention are administered to an individual already suffering from cancer, autoimmune disease, or infected with the virus of interest. Those in the incubation phase or the acute phase of the disease may be treated with the DR-binding peptides or immunogenic conjugates separately or in conjunction with other treatments, as appropriate.

In therapeutic applications, compositions comprising immunogenic compositions are administered to a patient in an amount sufficient to cure or at least partially arrest the symptoms of the disease and its complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on, e.g., the peptide composition, the manner of administration, the stage and severity of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing physician.

Therapeutically effective amounts of the immunogenic compositions of the present invention are in t;he general range of immunogenically effective dosages described below. These doses may be followed by boosting dosages pursuant to a boosting regimen

over weeks to months depending upon the patient's response and condition by measuring specific HTL activity in the patient's blood.

It must be kept in mind that the compositions of the present invention may generally be employed in serious disease states, that is, life-threatening or potentially life-threatening situations. In such cases, in view of the minimization of extraneous substances and the relative nontoxic nature of the conjugates, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these compositions.

For prophylactic use, administration should be given to risk groups. For example, protection against malaria, hepatitis, or AIDS may be accomplished by prophylactically administering compositions of the invention, thereby increasing immune capacity. Therapeutic administration may begin at the first sign of disease or the detection or surgical removal of tumors or shortly after diagnosis in the case of acute infection. This is followed by boosting doses until at least symptoms are substantially abated and for a period thereafter. In chronic infection, loading doses followed by boosting doses may be required.

Treatment of an infected individual with the compositions of the invention may hasten resolution of the infection in acutely infected individuals. For those individuals susceptible (or predisposed) to developing chronic infection the compositions are particularly useful in methods for preventing the evolution from acute to chronic infection. Where the susceptible individuals are identified prior to or during infection, for instance, as described herein, the composition can be targeted to them, minimizing need for administration to a larger population.

The peptide mixtures or conjugates can also be used for the treatment of chronic infection and to stimulate the immune system to eliminate virus-infected cells in carriers. It is important to provide an amount of immuno-potentiating peptide in a formulation and mode of administration sufficient to effectively stimulate helper T cell response. Immunizing doses followed by boosting doses at established intervals, e.g., from one to four weeks, may be required, possibly for a prolonged period of time to effectively immunize an individual. In the case of chronic infection, administration should continue until at least clinical symptoms or laboratory tests indicate that the viral infection has been eliminated or substantially abated and for a period thereafter.

The pharmaceutical compositions for therapeutic or prophylactic treatment are intended for parenteral, topical, oral or local administration. Typically, the pharmaceutical compositions are administered parenterally, e.g., intravenously, intrathecally,

subcutaneously, intradermally, or intramuscularly. Because of the ease of administration, the vaccine compositions of the invention are particularly suitable for oral administration. Thus, the invention provides compositions for parenteral administration which comprise a solution of the peptides or conjugates dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used, e.g., water, buffered water, 0.9% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilized by conventional, well known sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

The concentration of DR and/or CTL stimulatory peptides of the invention in the pharmaceutical formulations can vary widely, i.e., from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

The peptides or conjugates of the invention may also be administered via liposomes, which serve to target the conjugates to a particular tissue, such as lymphoid tissue, or targeted selectively to infected cells, as well as increase the half-life of the peptide composition. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. In these preparations the peptide to be delivered is incorporated as part of a liposome, alone or in conjunction with a molecule which binds to, e.g., a receptor prevalent among lymphoid cells, such as monoclonal antibodies which bind to the CD45 antigen, or with other therapeutic or immunogenic compositions. Thus, liposomes filled with a desired peptide or conjugate of the invention can be directed to the site of lymphoid cells, where the liposomes then deliver the selected therapeutic/immunogenic peptide compositions. Liposomes for use in the invention are formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of, e.g., liposome size, acid lability and stability

of the liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka, *et al., Ann. Rev. Biophys. Bioeng.* 9, 467 (1980), U.S. Patent Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369, incorporated herein by reference.

For targeting to the immune cells, a ligand to be incorporated into the liposome can include, e.g., antibodies or fragments thereof specific for cell surface determinants of the desired immune system cells. A liposome suspension containing a peptide or conjugate may be administered intravenously, locally, topically, etc. in a dose which varies according to, inter alia, the manner of administration, the conjugate being delivered, and the stage of the disease being treated.

Alternatively, DNA or RNA encoding one or more DR peptides (and optionally, a polypeptide containing one or more CTL epitopes or antibody inducing epitopes) may be introduced into patients to obtain an immune response to the polypeptides which the nucleic acid encodes. This approach is described, for instance, in Wolff *et. al.*, *Science* 247:1465 (1990) as well as U.S. Patent Nos. 5,580,859; 5,589,466; 5,804,566; 5,739,118; 5,736,524; 5,679,647; WO 98/04720; and in more detail below. Examples of DNA-based delivery technologies include "naked DNA", facilitated (bupivicaine, polymers, peptide-mediated) delivery, cationic lipid complexes, and particle-mediated ("gene gun") delivery.

A preferred means of administering nucleic acids encoding the peptides of the invention uses minigene constructs encoding one or multiple epitopes of the invention. The use of multi-epitope minigenes is described below and in, *e.g.* An, L. and Whitton, J. L., *J. Virol.* 71:2292, 1997; Thomson, S. A. *et al., J. Immunol.* 157:822, 1996; Whitton, J. L. *et al., J. Virol.* 67:348, 1993; Hanke, R. *et al., Vaccine* 16:426, 1998. For example, a multi-epitope DNA plasmid encoding nine dominant HLA-A*0201- and A11-restricted epitopes derived from the polymerase, envelope, and core proteins of HBV and HIV, the PADRE™ universal helper T cell (HTL) epitope, and an ER-translocating signal sequence was engineered. Immunization of HLA transgenic mice with this plasmid construct resulted in strong CTL induction responses against the nine epitopes tested, similar to those observed with a lipopeptide of known immunogenicity in humans, and significantly greater than immunization in oil-based adjuvants. Moreover, the immunogenicity of DNA-encoded epitopes *in vivo* correlated with the *in vitro* responses of specific CTL lines against target cells transfected with the DNA plasmid.

For example, to create a DNA sequence encoding the selected epitopes (minigene) for expression in human cells, the amino acid sequences of the epitopes may be reverse translated. A human codon usage table can be used to guide the codon choice for each amino acid. These epitope-encoding DNA sequences may be directly adjoined, so that when translated, a continuous polypeptide sequence is created. To optimize expression and/or immunogenicity, additional elements can be incorporated into the minigene design. Examples of amino acid sequences that could be reverse translated and included in the minigene sequence include: HLA class I epitopes, HLA class II epitopes, a ubiquitination signal sequence, a leader sequence, and/or an endoplasmic reticulum targeting signal. In addition, HLA presentation of CTL and HTL epitopes may be improved by including synthetic (*e.g.* poly-alanine) or naturally-occurring flanking sequences adjacent to the CTL or HTL epitopes.

The minigene sequence may be converted to DNA by assembling oligonucleotides that encode the plus and minus strands of the minigene. Overlapping oligonucleotides (30-100 bases long) may be synthesized, phosphorylated, purified and annealed under appropriate conditions using well known techniques. The ends of the oligonucleotides can be joined, for example, using T4 DNA ligase. This synthetic minigene, encoding the epitope polypeptide, can then be cloned into a desired expression vector.

Standard regulatory sequences well known to those of skill in the art are preferably included in the vector to ensure expression in the target cells. Several vector elements are desirable: a promoter with a down-stream cloning site for minigene insertion; a polyadenylation signal for efficient transcription termination; an *E. coli* origin of replication; and an *E. coli* selectable marker (*e.g.* ampicillin or kanamycin resistance). Numerous promoters can be used for this purpose, *e.g.*, the human cytomegalovirus (hCMV) promoter. *See, e.g.*, U.S. Patent Nos. 5,580,859 and 5,589,466 for other suitable promoter sequences.

Additional vector modifications may be desired to optimize minigene expression and immunogenicity. In some cases, introns are required for efficient gene expression, and one or more synthetic or naturally-occurring introns could be incorporated into the transcribed region of the minigene. The inclusion of mRNA stabilization sequences and sequences for replication in mammalian cells may also be considered for increasing minigene expression.

Once an expression vector is selected, the minigene is cloned into the polylinker region downstream of the promoter. This plasmid is transformed into an

appropriate *E. coli* strain, and DNA is prepared using standard techniques. The orientation and DNA sequence of the minigene, as well as all other elements included in the vector, are confirmed using restriction mapping and DNA sequence analysis. Bacterial cells harboring the correct plasmid can be stored as a master cell bank and a working cell bank.

In addition, immunostimulatory sequences (ISSs or CpGs) appear to play a role in the immunogenicity of DNA vaccines. These sequences may be included in the vector, outside the minigene coding sequence, if desired to enhance immunogenicity.

In some embodiments, a bi-cistronic expression vector which allows production of both the minigene-encoded epitopes and a second protein (included to enhance or decrease immunogenicity) can be used. Examples of proteins or polypeptides that could beneficially enhance the immune response if co-expressed include cytokines (*e.g.*, IL-2, IL-12, GM-CSF), cytokine-inducing molecules (*e.g.*, LeIF) or costimulatory molecules. Helper (HTL) epitopes can be joined to intracellular targeting signals and expressed separately from expressed CTL epitopes; this allows direction of the HTL epitopes to a cell compartment different than that of the CTL epitopes. If required, this could facilitate more efficient entry of HTL epitopes into the HLA class II pathway, thereby improving CTL induction. In contrast to HTL or CTL induction, specifically decreasing the immune response by co-expression of immunosuppressive molecules (*e.g.* TGF-β) may be beneficial in certain diseases).

Therapeutic quantities of plasmid DNA can be produced for example, by fermentation in *E. coli*, followed by purification. Aliquots from the working cell bank are used to inoculate growth medium, and grown to saturation in shaker flasks or a bioreactor according to well known techniques. Plasmid DNA can be purified using standard bioseparation technologies such as solid phase anion-exchange resins supplied by QIAGEN, Inc. (Valencia, California). If required, supercoiled DNA can be isolated from the open circular and linear forms using gel electrophoresis or other methods.

Purified plasmid DNA can be prepared for injection using a variety of formulations. The simplest of these is reconstitution of lyophilized DNA in sterile phosphate-buffer saline (PBS). This approach, known as "naked DNA," is currently being used for intramuscular (IM) administration in clinical trials. To maximize the immunotherapeutic effects of minigene DNA vaccines, an alternative method for formulating purified plasmid DNA may be desirable. A variety of methods have been described, and new techniques may become available. Cationic lipids can also be used in the

formulation (*see, e.g.*, as described by WO 93/24640; Mannino & Gould-Fogerite, *BioTechniques* 6(7): 682 (1988); U.S. Pat No. 5,279,833; WO 91/06309; and Felgner, *et al.*, *Proc. Nat'l Acad. Sci. USA* 84:7413 (1987). In addition, glycolipids, fusogenic liposomes, peptides and compounds referred to collectively as protective, interactive, non-condensing (PINC) could also be complexed to purified plasmid DNA to influence variables such as stability, intramuscular dispersion, or trafficking to specific organs or cell types.

In vitro assays can be used as functional assays for expression of HTL epitopes. For example, the plasmid DNA is introduced into a mammalian cell line that is a suitable presenter of HTL epitopes.. The transfection method used will be dependent on the final formulation. Electroporation can be used for "naked" DNA, whereas cationic lipids allow direct *in vitro* transfection. A plasmid expressing green fluorescent protein (GFP) can be co-transfected to allow enrichment of transfected cells using fluorescence activated cell sorting (FACS). The cells may then be assayed for the ability to elicit an HTL response using methods known in the art (*see, e.g.*, Alexander *et al.*, *Immunity* 1:751-761, 1994)

In vivo immunogenicity is a second approach for functional testing of minigene DNA formulations which include both CTL and HTL epitopes. Transgenic mice expressing appropriate human HLA proteins are immunized with the DNA product. The dose and route of administration are formulation dependent (*e.g.*, IM for DNA in PBS, IP for lipid-complexed DNA). Twenty-one days after immunization, splenocytes are harvested and restimulated for 1 week in the presence of peptides encoding each epitope being tested. For CTL effector cells, assays are conducted for cytolysis of peptide-loaded, chromium-51 labeled target cells using standard techniques. Lysis of target cells sensitized by HLA loading of peptides corresponding to minigene-encoded epitopes demonstrates DNA vaccine function for *in vivo* induction of CTLs.

Alternatively, the nucleic acids can be administered using ballistic delivery as described, for instance, in U.S. Patent No. 5,204,253. Using this technique, particles comprised solely of DNA are administered. In a further alternative embodiment, DNA can be adhered to particles, such as gold particles.

For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers

previously listed, and generally 10-95% of active ingredient, that is, one or more conjugates of the invention, and more preferably at a concentration of 25%-75%.

For aerosol administration, the peptides are preferably supplied in finely divided form along with a surfactant and propellant. Typical percentages of conjugates are 0.01%-20% by weight, preferably 1%-10%. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. The surfactant may constitute 0.1%-20% by weight of the composition, preferably 0.25-5%. The balance of the composition is ordinarily propellant. A carrier can also be included, as desired, as with, e.g., lecithin for intranasal delivery.

In another aspect the present invention is directed to vaccines which contain as an active ingredient an immunogenically effective amount of an immunogenic DR peptide or a CTL\DR peptide conjugate or nucleic acid encoding them as described herein. The conjugate(s) may be introduced into a host, including humans, linked to its own carrier or as a homopolymer or heteropolymer of active peptide units. Such a polymer has the advantage of increased immunological reaction and, where different peptides are used to make up the polymer, the additional ability to induce antibodies and/or CTLs that react with different antigenic determinants of the virus or tumor cells. Useful carriers are well known in the art, and include, e.g., thyroglobulin, albumins such as bovine serum albumin, tetanus toxoid, polyamino acids such as poly(lysine:glutamic acid), hepatitis B virus core protein, hepatitis B virus recombinant vaccine and the like. The vaccines can also contain a physiologically tolerable (acceptable) diluent such as water, phosphate buffered saline, or saline, and further typically include an adjuvant. Adjuvants such as incomplete Freund's adjuvant, aluminum phosphate, aluminum hydroxide, or alum are materials well known in the art. And, as mentioned above, CTL responses can be primed by conjugating peptides of the invention to lipids, such as P_3CSS. Upon immunization with a peptide composition as described herein, via injection, aerosol, oral, transdermal or other route, the immune system of the host responds to the vaccine by producing large amounts of CTLs and/or HTLs specific for the desired antigen, and the host becomes at least partially immune to later infection, or resistant to developing chronic infection.

Vaccine compositions containing the DR peptides of the invention are administered to a patient susceptible to or otherwise at risk of disease, such as viral infection or cancer in an amount that will elicit an immune response against the antigen and thus enhance the patient's own immune response capabilities.

A therapeutically effective amount and an amount used for vaccine of a peptide disclosed herein is defined to be an "immunogenically effective dose." In this use, the precise amounts again depend on the patient's state of health and weight, the mode of administration, the nature of the formulation, etc., but generally occur in a unit dosage range where the lower value is about 1, 5, 50, 500, or 1000 μg and the higher value is about 10,000; 20,000; 30,000; or 50,000 μg. Dosage values for a human typically range from about 500 μg to about 50,000 μg per 70 kilogram patient.

In some instances it may be desirable to combine the peptide vaccines of the invention with vaccines which induce neutralizing antibody responses to the virus of interest, particularly to viral envelope antigens. For instance, PADRE peptides can be combined with hepatitis vaccines to increase potency or broaden population coverage. Suitable hepatitis vaccines that can be used in this manner include, Recombivax HB® (Merck) and Engerix-B (Smith-Kline).

For therapeutic or immunization purposes, the peptides of the invention can also be expressed by attenuated viral hosts, such as vaccinia or fowlpox. This approach involves the use of vaccinia virus as a vector to express nucleotide sequences that encode the peptides of the invention. Upon introduction into an acutely or chronically infected host or into a non-infected host, the recombinant vaccinia virus expresses the immunogenic peptide, and thereby elicits a host CTL response. Vaccinia vectors and methods useful in immunization protocols are described in, e.g., U.S. Patent No. 4,722,848, incorporated herein by reference. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover *et al., Nature* 351, 456-460 (1991)) which is incorporated herein by reference. A wide variety of other vectors useful for therapeutic administration or immunization of the peptides of the invention, e.g., Salmonella typhi vectors and the like, will be apparent to those skilled in the art from the description herein.

The peptide epitopes of the invention may be administered to antigen presenting cells (APCs), preferably dendritic cells, *ex vivo*, as well. In a preferred embodiment, responses to a particular pathogen (infectious agent or tumor antigen) are induced by pulsing APCs with the peptide epitope and subsequently administering the pulsed

APC, wherein the cells then present the peptide *in vivo*. The pulsed APCs may be administered in vivo as described above for the peptides.

Peptides epitopes of the invention may also be used in conjunction with CTL epitopes to elicit CTL *ex vivo* as well. The resulting CTL can be used to treat infections or tumors. Ex vivo CTL responses to a particular pathogen are induced by incubating in tissue culture the patient's CTL precursor cells together with a source of antigen-presenting cells and the appropriate immunogenic peptide epitopes. After an appropriate incubation time *typically 1-4 weeks) in which the CTL precursor cells are activated and expanded into effector CTL, the cells are infused back into the patient, where they will destroy their specific target cell (an infected cell or a tumor cell).

The peptides of this invention may also be used to make monoclonal antibodies. Such antibodies may be useful as potential diagnostic or therapeutic agents.

The peptides may also find use as diagnostic reagents. For example, a peptide of the invention may be used to determine the susceptibility of a particular individual to a treatment regimen which employs the peptide or related peptides, and thus may be helpful in modifying an existing treatment protocol or in determining a prognosis for an affected individual. In addition, the peptides may also be used to predict which individuals will be at substantial risk for developing chronic infection.

Examples

Materials and Methods

Cells. The following Epstein-Barr virus (EBV) transformed homozygous cell lines were used as sources of human HLA class II molecules: LG2 [DRB1c0101 (DR1)1; GM3107 [DRB50101 (DR2w2a)]; MAT (DRB10301 (DR3)1; PREISS [DRB10401 (DR4w4)1; BIN40 [DRB10404 (DR4w14)1; SWEIG [DRB11101 (DR5w11)]; PITOUT [DRB10701 (DR7)] (a); KT3 [DRB10405 (DR4w15)]; Herluf [DRB11201 (DR5w12)]; HO301 [DRB11302 (DR6w19)]; OLL [DRB10802 (DR8w2)]; and HTC9074 [DRB10901 (DR9), supplied as a kind gift by Dr. Paul Harris, Columbia University]. In some instances, transfected fibroblasts were used: L466.1 [DRB11501 (DR2w2b)]; TR81.19 [DRB30101 (DR52a)]; and L257.6 [DRB40101 (DRw53)]. (Valli, *et al. J. Clin. Invest.* 91:616 (1993). Cells were maintained *in vitro* by culture in RPMI 1640 medium supplemented with 2mM L-glutamine [GIBCO, Grand Island, NY], 50μM 2-ME, and 10% heat-inactivated FCS [Irvine Scientific, Santa Ana, CA]. Cells were also supplemented with 100 μg/ml of

streptomycin and 10OU/ml of penicillin [Irvine Scientific]. Large quantities of cells were grown in spinner cultures.

Cells were lysed at a concentration of 10^8 cells/ml in PBS containing 1% NP-40 [Fluka Biochemika, Buchs, Switzerland], 1mM PMSF [CalBioChem, La Jolla, CA], 5mM Na-orthovanadate, and 25mM iodoacetamide [Sigma Chemical, St. Louis, Mo]. The lysates were cleared of debris and nuclei by centrifugation at 10,000 x *g* for 20 min.

Affinity purification of HLA-DR molecules. Class II molecules were purified by affinity chromatography as previously described (Sette, *et al. J. Immunol.* 142:35 (1989) and Gorga, *et al. J. Biol. Chem.* 262:16087 (1987)) using the mAb LB3.1 coupled to Sepharose 4B beads. Lysates were filtered through 0.8 and 0.4 μM filters and then passed over the anti-DR column, which were then washed with 15-column volumes of 10mM TRIS in 1% NP-40, PBS and 2-column volumes of PBS containing 0.4% n-octylglucoside. Finally, the DR was eluted with 5OmM diethylamine in 0.15M NaCl containing 0.4% n-octylglucoside, pH 11.5. A 1/25 volume of 2.0M Tris, pH 6.8, was added to the eluate to reduce the pH to ~8.0, and then concentrated by centrifugation in Centriprep 30 concentrators at 2000 rpm (Amicon, Beverly, MA).

Class II peptide-binding assays. A panel of 13 different specific DR-peptide assays were utilized in the present study. These assays were chosen as to be representative of the most common DR alleles. Table I lists for each DR antigen, the representative allelic product utilized, the cell line utilized as a source of DR, and the radiolabled probe utilized in the assay. Purified human class II molecules [5 to 500 nM] were incubated with various unlabeled peptide inhibitors and 1-10 nM ^{125}I-radiolabeled probe peptides for 48h in PBS containing 5% DMSO in the presence of a protease inhibitor cocktail. The radiolabeled probes used were HA Y307-319 (DR1), Tetanus Toxoid[TT] 830-843 (DR2w2a, DR5w111, DR7, DR8w2, DR8w3, DR9), MBP Y85-100 (DR2w2b), TT1272-1284 (DR52a), MT 65 kD Y3-13 with Y7 substituted with F for DR3, a non-natural peptide with the sequence YARFQSQTTLKQKT (DR4w4, DR4w15, DRw53) (Valli, *et al. supra*), and for DR5w12, a naturally processed peptide eluted from the cell line C1R, EALIHQLINPYVLS (DR5w12) and 650.22 peptide, (TT 830-843 A ® S836 analog), for DR6w19.

Radiolabeled peptides were iodinated using the chloramine-T method. Peptide inhibitors were typically tested at concentrations ranging from 1201 μg/ml to 1.2 ng/ml. The data were then plotted and the dose yielding 50% inhibition (IC50) was measured. In appropriate stoichiometric conditions, the IC50 of an unlabeled test peptide to

the purified DR is a reasonable approximation of the affinity of interaction (Kd). Peptides were tested in two to four completely independent experiments. The final concentrations of protease inhibitors were: 1mM PMSF, 1.3nM 1.10 phenanthroline, 73 μM pepstatin A, 8mM EDTA, and 200 μM N alpha-p-tosyl-L-lysine chloromethyl ketone (TLCK) [All protease inhibitors from CalBioChem, La Jolla, CA]. Final detergent concentration in the incubation mixture was 0.05% Nonidet P-40. Assays were performed at pH 7.0 with the exception of DR3, which was performed at pH 4.5, and DRw53, which was performed at pH 5.0. The pH was adjusted as previously described (Sette, *et al. J. Immunol.* 148:844 (1992)).

Class II peptide complexes were separated from free peptide by gel filtration on TSK2000 columns (TosoHaas 16215, Montgomeryville, PA), and the fraction of bound peptide calculated as previously described (Sette, *et al.*, (1989) *supra*). In preliminary experiments, the DR prep was titered in the presence of fixed amounts of radiolabeled peptides to determine the concentration of class II molecules necessary to bind 10-20% of the total radioactivity. All subsequent inhibition and direct binding assays were the performed using these class II concentrations.

DRB1 specificity of DR4w15, DR6w19, DR8w2, DR8w3, and DR9 assays.

Because the antibody used for purification is α-chain specific, β1 molecules are not separated from β3 (and/or β4 and β5) molecules. Development and validation of assays in regard with DRβ chain specificity has been described in detail elsewhere for many of the DR alleles listed above (108). Herein we describe for the first time DR4w15, DR6w19, DR8w2, DR8w3, and DR9 assays. Experiments addressing the β chain specificity of these new assays are described in the present section.

DR4w15. The β4 product DRw53 is co-expressed with DR4w15 and the determination of the specificity of the DR4w15 binding assay is complicated in that the same radiolabeled ligand is used for both the DR4w15 and DRw53 binding assays. Since typically β1 chains are expressed at 5-10 fold higher levels than other β chains, and all binding assays are performed utilizing limiting DR amounts, it would be predicted that the dominant specificity detected in the assay would be DR4w15. To verify that this was indeed the case, the binding pattern of a panel of 58 different synthetic peptides in the putative DR4w15 specific assay with that obtained in a DRw53 specific assay (which uses a DRw53 fibroblast as the source of class II molecules). Two very distinct binding patterns were noted, and in

several instances, a peptide bound to one DR molecule with high affinity, and did not bind to the other (data not shown).

DR6w19. The DR6w19 assay utilizes as the source of class II molecules the EBV transformed homozygous cell line H0301, which co-expresses DRB30301 (DR52a). While the radiolabeled ligand used in the DR6w19 assay is different than that used for the DR52a assay, the ligand is related (i.e., is a single substitution analog) to a high affinity DR52a binder. As was done in the case of DR4w15, the specificity of the assay was investigated by analyzing the binding capacity of a panel of naturally occurring peptides for DR6w19 and DR52a. The two assays demonstrated completely different binding specificities. For example, in terms of relative binding, TT 1272-1284 binds 63-fold better in the DR52a assay than in the DR6w19 assay. Conversely, the Invariant chain peptide binds 189-fold better in the DR6w19 assay. In conclusion, these data demonstrated that the binding of the radiolabeled peptide 650.22 to purified class II MHC from the H0301 cell line is specific for DR6w19.

DR8w2 and DR8w3. The β1 specificity of the DR8w2 and DR8w3 assays is obvious in that no β3 (and/or B4 and β5) molecule is expressed.

DR9. The specificity of DR9 assay is inferred from previous studies which have shown that the TT 830-843 radiolabeled probe peptide does not bind to DRw53 molecules (Alexander, *et al.*, *Immunity 1:751* (1994)).

Results

DR binding affinity of antigenic peptides recognized by DR restricted T cells

To define a threshold DR binding affinity, to be considered as biologically significant, we compiled the affinities of a panel of 32 reported instances of DR restriction of a given T cell epitope. In approximately half of the cases, DR restriction was associated with affinities of less than 100 nM, and in the other half of the instances, with IC50% in the 100-1000 nM range. Only in 1 out of 32 cases (3.1%) DR restriction was associated with IC50% of 1000 nM or greater. It was noted that this distribution of affinities differs from what was previously reported for HLA class I epitopes, where a vast majority of epitopes bound with IC50% of 50 nM or less (Sette, *et al.*, JI, 1994). This relatively lower affinity of class II restricted epitope interactions might explain why activation of class II restricted T cells in general requires more antigen relative to class I restricted T cells.

In conclusion, this analysis suggested that 1000 nM may be defined as an affinity threshold associated with immunogenicity in the context of DR molecules, and for this reason a suitable target for our studies.

P1 and P6 anchors are necessary but not sufficient for DRB10401 binding

Several independent studies have pointed to a crucial role in DRB10401 binding of a large aromatic or hydrophobic residue in position 1, near the N-terminus of the peptide and of a 9-residue core region (residues 1 through 9). In addition, an important role has been demonstrated for the residue in position six (P6) of this 9-residues core region. Short and/or hydrophobic residues were in general preferred in this position (O'Sullivan, *et al.*, JI 147:2663, 1991; Sette, *et al.*, JI 151:3163, 1993; Hammer, *et al.*, Cell 74:197, 1993 and Marshall, *et al.*, JI 154:5927, 1995).

In the present set of experiments, a library of 384 peptides was analyzed for DRB10401 binding capacity and screened for the presence of the P1-P6 motif (that is, F, W, Y, L, I, V or M in P1 and S, T, C, A, P, V, I, L or M in P6, at least 9 residues apart from the peptide C-terminus. This set of 384 peptides contained a total of 80 DR4w4 binders (specifically 27 good binders [IC50 of 100 nM or less], and 53 intermediate binders [IC50 of the 100-1000 range]. Seventy-seven out of the 80 DR4w4 binders (96%) carried the P1-P6 motif. However, it should be noted that most non-DR4w4 binding peptides also contained the P1-P6 motif. Of 384 peptides included in our database, only 125 were "P1-P6 negative." Only three of them (6%) bound appreciably to purified DR4w4 as opposed to 77/259 (30%) of the "P1-P6 positive" peptides. Therefore, these results demonstrate that presence of suitable P1 and P6 anchors are necessary but not sufficient for DRB10401 binding.

A detailed map of DRB10401 peptide interactions

Next, for each P1-P6 aligned core region, in analogy with what the strategy previously utilized to detail peptide class I interactions the average binding affinity of peptides carrying a particular residue, relative to the remainder of the group, were calculated for each position. Following this method a table of average relative binding (ARB) values was compiled. This table also represents a map of the positive or negative effect of each of the 20 naturally occurring amino acids on DRB10401 binding capacity when occupying a particular position, relative to the main P1-P6 anchors (Figure 1).

Variations in ARB values greater than four fold (ARB ³ 4 or £ 0.25) were arbitrarily considered significant and indicative of secondary effects of a given residue on

DR-peptide interactions. Most secondary effects were associated with positions 4, 7, and 9. These positions correspond to secondary anchors engaging shallow pockets on the DR molecule. In addition, significant secondary effects were detected for M in position 3 (ARB = 12.8) T in position 3 (ARB = 4.34) and I in position 5 (ARB = 4.4).

Development of a DRB10401 specific algorithm

Next, the ARB table was utilized to develop a DRB10401 specific algorithm. In order to predict 0401 binding propensity, each aligned P1-P6 sequence was scored by multiplying, for each position, the ARB value of the appropriate amino acid. According to this procedure, a numerical "algorithm score" was derived. If multiple P1-P6 alignments were possible, binding scores were calculated for each one and the best score was selected. The efficacy of this method in predicting 0401 binding capacity is shown in Table IIa.

Considering only peptides with algorithm scores above -17.00 narrowed the set of predicted peptides to 156. This set still contained 72 out of 80 (90%) of the total high or intermediate DR binders. Raising the cut-off to an algorithm score of -16.44 or higher still allowed identification of 60 out of 80 (75%) of the DR4w4 binding peptides. Of the whole 107 peptide set, twenty-five of them were either good or intermediate binders. In other words, as expected, increasing the algorithm score stringency predicted a smaller fraction of the total binders present in the set, but at the same time less false positive peptides were identified.

Blind test of the predictive power of the DRB10401 specific algorithm

To verify that the predictive capacity of our algorithm was not merely a reflection of having utilized the same data set to test and define the algorithm itself, we further examined its efficacy in a blind prediction test. For this scope we utilized data from an independent set of 50 peptides, whose binding affinities were known, but that had not been utilized in the derivation of the algorithm. As shown in Table IIb, the algorithm was effective in predicting DR4w4 binding capacity of this independent peptide set. The algorithm score of -17.00 identified a total 18 peptides. This set contained 3/3 (100%) of all good binders, and 8/11 (70%) of all intermediate binders in the entire test set of 50 peptides. Increasing the cut-off value to -16.44, identified a set of nine peptides. Seven of them (78%) were either good or intermediate binders. This set contained 7 out of 14 (50%) of the

binders contained in the blind prediction peptide set. In conclusion, these data supports the validity of the DR4w4 specific algorithm described above.

Detailed maps of DRB10401, DRB10101, and DRB10701 peptide binding specificities

Next, we analyzed the binding to purified DR1 and DR7 molecules for the same set of 384 peptides utilized to define the DR4w4 algorithm. It was found that this set contained 120 and 59 binders for the DR1 and DR7 alleles, respectively. A total of 158 peptides were capable of binding either DR1, DR4w4 or DR7. A large fraction of them (73/158; 46%) were also degenerate binders, which bound two or more of the three alleles thus far considered. Furthermore, we also found that more than 90% of the DR1 or DR7 good and intermediate binders carried the P1-P6 motif. Most importantly, 72 out of 73 (99%) degenerate DR binders carried this motif (data not shown). In conclusion, this analysis suggests that P1-P6 based algorithms might be utilized to effectively predict degenerate DR binders.

In analogy with what was described above for DR4w4 molecules, specific algorithms were designed for the DR1 and DR7 alleles. Figures 2A and 2B detail the allele specific maps defined according to this method.

As in the case of DRB10401, most secondary effects were concentrated in positions 4, 7 and 9. Position 4 was especially prominent in the case of DR1, while position 7 was the most prominent secondary anchor for DR7. Specific algorithms were developed based on these maps, and it was found that the cut-off values necessary to predict 75% or 90% of the binders were -19.32 and -20.28 for DR1, and 20.91 and -21.63 for DR7, respectively. Depending on the particular allele or cut off value selected, 40 to 60% of the predicted peptides were in fact good or intermediate binders (data not shown).

Development of a DR1-4-7 combined algorithm

Finally, we examined whether a combined algorithm would allow to predict degenerate binders. For this purpose, the sequences of the 384 peptides in our database were simultaneously screened with the three (DR1, 4w4, and 7) specific algorithms. It was found that an even 100 peptides were predicted (using the 75% cut off) to bind either two or three of the alleles considered. This set contained 59 out of 73 (81%) of the peptides which were in fact capable of degenerate 1-4-7 binding (defined as the capacity to bind to more than one of the DR1, 4w4 or 7 alleles) (Table III).

Definition of a target set of DR specificities, representative of the world population

The data presented in the preceding sections illustrates how peptides capable of binding multiple DR alleles can be identified by the use of a combined "1-4-7" algorithm. Next, we wished to examine whether the peptides exhibiting degenerate 1-4-7 binding behavior would also bind other common DR types as well. As a first step in our experimental strategy, we sought to define a set of target DR types representative of a large (³ 80%) fraction of the world population, irrespective of the ethnic population of origin. For this purpose, seven additional DR antigens were considered. For each one of the DR antigens considered in this study, (including DR1, 4 and 7), the estimated frequency in various ethnicities, according to the most recent HLA workshop (11th, 1991) is shown in Table IVa, together with the main subtypes thus far identified.

For the purpose of measuring peptide binding affinity to the various DR molecules, one representative subtype for each DR antigen was chosen (Table I). It should be noted that for most antigens, either one subtype is by far the most abundant, or alternatively a significant degree of similarity in the binding pattern displayed by the different, most abundant subtypes of each DR antigen is likely to exist (see comments column of Table IVb). One exception to this general trend is represented by the DR4 antigen, for which significant differences in peptide specificity between the 0401 and 0405 have been reported. Since both alleles are quite frequent (in Caucasians and Orientals, respectively) we included both DR 0401 and 0405 in the set of representative DR binding assays.

Our set of representative assays is mostly focused on allelic products of the gene, because these molecules appear to be the most abundantly expressed, serve as the dominant restricting element of most human class III responses analyzed thus far, and accurate methods for serologic and DNA typing most readily available. However, we have also considered in our analysis assays representative of DRB3/4/5 molecules (Table IVc). These molecules serve as a functional restriction element, and their peptide binding specificity has been previously shown to have certain similarities to the specificity of several common DRβ_1 allelic products.

A general strategy for prediction of DR-degenerate binders.

To test whether the 1-4-7 combined algorithm would also predict degenerate binding to other common DR types, we measured the capacity of three different groups of synthetic peptides to bind the panel of purified HLA DR molecules. The three different peptide sets were: A) 36 peptides which did not score positive in the combined 1-4-7 algorithm (non-predictions), B) 36 peptides which did score positive for the 1-4-7 algorithm, at the 75% cut off level, but had been found upon actual testing not to be degenerate 1-4-7 binders ("wrong" predictions), and C) 29 peptides which scored positive in the 1-4-7 algorithm, and also proved upon experimental testing, to be actual 1-4-7 degenerate binders (correct predictions). The results of this analysis are shown in Table V.

Within the set of "non-predictions" peptides (Table Va) only 3 out of 34 (9%) bound at least two of the DR1, 4w4 or 7 molecules. Interestingly, 2 (1136.04 and 1136.29) out of 3 of these peptides were also rather crossreactive, and bound additional DR types (DR2w2 β2, DR4w15, 5w11 and 8w2 in the case of 1136.04, and 2w2 β2, 4w15, 9 and 5w12 in the case of 1136.29). Peptides from the "wrong predictions" peptide set (Table V5), by definition bound at the most only one of the DR1, 4w4 or DR7 molecules, and were also poorly degenerate or other DR types with
only two peptides (1136.22 and 1188.35) binding a total of three DR molecules. Within this peptide set, no peptide bound four or more of the DR molecules tested (data not shown).

These results are contrasted by data obtained with the peptide set corresponding to peptides which were first predicted by the use of the combined 1, 4, 7 algorithm, and then experimentally found to be degenerate DR1-4-7 binding. Fourteen out of 29 peptides tested (48%) bound a total of five or more alleles. Four of them were remarkably degenerate (1188.16, 1188.32, 1188.34 and F107.09) and bound a total of nine out of the 11 DR molecules tested. In conclusion, these results suggest that a strategy based on the sequential use of a combined DR1, 4, 7 algorithm and quantitative DR1, 4, 7 binding assays can be utilized to identify broadly crossreactive DR binding peptides.

Definition of the HLA-DR 1-4-7 supertype

The data presented above also suggested that several common DR types are characterized by largely overlapping peptide binding repertoires. When this issue was analyzed in more detail, by analyzing the binding pattern of the thirty-two peptides from Table Va and b which were actual DR1-4-7 degenerate binders. Thirty-one of them (97%) bound DR1, 22 (69%) DR4w4 and 21 (66%) DR7. These files are contrasted with the low

percentages of binding observed amongst the remainder non-degenerate binding peptides (17/67 (25%), 8/67 (12%) and 7/67 (10%), for DR1, 4w4 and 7, respectively) (Table VII).

Interestingly, a large fraction of the 1-4-7 degenerate binders also bound certain other common DR types. Sixteen (50%) bound DR2w2a, 18 (56%) DR6w19, 18 (56%) DR2w2b and 20 (62%) DR9. In all cases, the frequency of binding in the non-1-4-7 degenerate peptide set was much lower (Table VIII).

Significant, albeit lower, frequencies of cross reactivity were noted also for DR4w15, DR5w11, and DR8w2 (in the 28 to 37% range). Finally, negligible levels of cross reactivity were observed in the case of DR3 and 5w12 and DR53. Further studies will address whether either of these two group of molecules (DR4w15, 5w11, and 8w2 on one hand, and DR3, DR53 and 5w12 on the other) might belong to different DR supertypes.

In conclusion, these data demonstrates that a large set of DR molecules encompassing DR1, 4w4, 2w2a, 2w2b, 7, 9 and 6w19 is characterized by largely overlapping peptide binding repertoires.

Discussion

In the present report we have analyzed the peptide binding specificity of a set of 13 different DR molecules, representative of DR types common among the worldwide population. Detailed maps of secondary anchors and secondary interactions have been derived for three of them (DR4w4, DR1 and DR7). Furthermore, we demonstrated that a set of at least seven different DR types share overlapping peptide binding repertoires; and consequently that broadly degenerate HLA DR binding peptides are a relatively common occurrence. This study also describes computerized procedures which should greatly assist in the task of identification of such degenerate peptides.

We would like to discuss the data in the context of our current understanding of peptide-class II interactions, as well as in the context of the recently described class I supermotifs. Finally, the potential implications of broadly degenerate class II epitopes for epitope based vaccine design should also be considered.

Firstly, our studies illustrate how the vast majority of the peptides binding with good affinity to DR4w4, DR1, DR7 and most of the other DR types analyzed in the current study (data not shown), are all characterized by a P1-P6 motif consistent with the one originally proposed by O'Sullivan, *et al.* Crystallographic analysis of DR1-peptide complexes revealed that the residues occupying these positions engage two complementary

pockets on the DR1 molecule, with the P1 position corresponding to the most crucial anchor residue and the deepest hydrophobic pocket. Our analysis also illustrates how other "secondary anchor" positions drastically influence in an allele-specific manner peptide binding capacity. Position 4 was found to be particularly crucial for DR1 binding, position 9 for DR4w4, and position 7 for DR7. These data are consistent with previous results which originally described such allele-specific anchors, and with crystallographic data which illustrates how these residues engage shallow pockets on the DR molecule.

Secondly, our studies illustrate how an approach based on alignment and calculation of average relative binding values of large peptide libraries allows definition of quantitative algorithms to predict binding capacity. The present study extends those observations to two other common HLA-DR types, and also illustrates how the combined use of the 1-4-7 algorithms can be of aid in identifying broadly degenerate DR binding peptides.

The data presented herein suggest that a group of common DR alleles, including at least DR1, DR2w2a, DR2w2b, DR4w4, DR6w19, DR7 and DR9 share a largely overlapping peptide repertoire. Degenerate peptide binding to multiple DR alleles, and recognition of the same epitope in the context of multiple DR types was originally described by Lanzavechia, Sinigallia's and Rothbard's groups. The present study provides a classification of alleles belonging to a main HLA-DR supertype (DR1-4-7-like) which includes DR1, DR2w2a, DR2w2b, DR4w4, DR7, DR9, DR6w19. On the basis of the data presented herein, at least two additional groups of alleles exist. The first group encodes for molecules with significant, albeit much reduced overlap with the 1-4-7-like supertype (DR4w15, 8w2, 5w11). The second group of alleles (5w12, 3w17, and w53) clearly has little repertoire association with the 1-4-7 supertype. In this context it is interesting to note that Hammer, *et al.* noted that good DR5w11 binding peptides are frequently characterized by positively charged P6 anchor (which would be poorly compatible) with the herein proposed 1-4-7 supermotif. It is also interesting to note that Sidney, *et al.* proposed that DR3w17 binds a set of peptides largely distinct from those bound by other common DR types. Future studies will have to determine whether any of the molecules listed above can be grouped in additional DR supertypes. Our group is currently investigating whether analysis of polymorphic residues lining the peptide binding pockets of DR can be utilized to aid in the classification and prediction of HLA DR supertypes.

We would like to comment on similarities and differences between the HLA DR supertype described herein and the recently described HLA class I supermotifs. Class I supermotifs are clear-cut and, as a rule, non-overlapping. Four of them have been described all approximately equally frequent amongst the worldwide population. By contrast, the repertoire defining the HLA DR supertype herein described is not clear-cut and overlaps, at least in part, with the repertoire of other alleles. It also appears that on the basis of the data presented in Tables I and IV, even if other DR supertypes exist, the DR1-4-7 is going to be by far the most abundantly represented worldwide.

Finally, we would like to point out the possible relevance of these data in terms of development of epitope based vaccines. Class II restricted HTL have been implicated in protection from, and termination of many important diseases. Inclusion of well defined class II epitopes in prophylactic or therapeutic vaccines may allow the immune response to focus towards conserved or subdominant epitopes, and avoid suppressive determinants. Based on the data presented herein (Table IV), the DR1-4-7 supertype would allow coverage in the 50 to 80% range, depending on the ethnicities considered. It is thus possible that broad and not ethnically biased population coverage could be achieved by considering a very limited number of peptide binding specificities.

Based on the results present above, the sequences of various antigens of interest were scanned for the presence of the DR 1-4-7 motifs. Peptides identified using this approach are broadly cross reactive, class II restricted T cell epitopes. Table VIII presents a listing of such peptides derived from various antigens and includes representive epitopes that bind one or more DR alleles at an IC_{50} of 1000 nM or less. The information in Table VIII includes the antigen from which the peptide was derived, and binding data expressed as IC_{50} values for the designated DR alleles as shown.

The above examples are provided to illustrate the invention but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the art. All publications, patents, and patent applications cited herein are hereby incorporated by reference for all purposes.

Table I

HLA-DR binding assays utilized in the present study.

Antigen	Representative Assay: Allele	Alias	Cell Line	Radiolabeled Probe	Ref.	Comments
DR1	DRB1*0101	(DR1)	LG2	HA Y307-319 [1]	(8)	01 is the most prevalent DR1 allele.
DR2	DRB1*1501	(DR2w2b)	L466.1	MBP 88-102Y [2]	(8)	0101 is the most prevalent DR2 allele.
DR3	DRB1*0301	(DR3w17)	MAT	MT 65kD Y3-13 analog [3]	(8)	01 is the most prevalent DR3 allele in most major populations. 01 and 02 are split fairly evenly in NA Blacks.
DR4	DRB1*0401	(DR4w4)	Preiss	Non-natural peptide YAR [4]	(8)	01 is the most prevalent DR4 allele.
	DRB1*0405	(DR4w15)	KT3	Non-natural peptide YAR	This paper	05 is the most prevalent DR4 allele in the Orient.
DR7	DRB1*0701	(DR7)	Pitout	TT 830-843 [5]	(8)	01/02 vary at 1 pos., which is outside the binding groove.
DR8	DRB1*0802	(DR8w2)	OLL	TT 830-843	This paper	02 is dominant in most major population groups. 02 and 03 have nearly identical binding specificities (J. Sidney and A. Sette, unpublished observations).
DR9	DRB1*0901	(DR9)	9074 (HID)	TT 830-843	This paper	DR9 splits are products of a silent mutation.
DR11	DRB1*1101	(DR5w11)	Swelg	TT 830-843	(8)	01 is the most prevalent DR11 allele, by far.
DR12	DRB1*1201	(DR5w12)	Herluf	CIR derived peptide [6]	(9)	01/02 are evenly distributed. These alleles differ at pos. 67, which does not appear strongly influence peptide binding.
DR13	DRB1*1302	(DR6w19)	HO301	650.22 (TT 830-843 analog) [7]	(10)	02 is slightly more prevalent overall than 01. These alleles vary at pos. 86(critical in determining the P1 anchor specificity.
DR51	DRB5*0101	(DR2w2a)	GM3107	TT 830-843 [5]	(8)	0101 is the most prevalent split.
DR53	DRB4*0101	(DR4, DR7, DR9)	L257.6	Non-natural peptide YAR [4]	(8)	0101 is essentially the only allele.

1) YPKYVKQNTLKLAT
2) VVHFFKNIVTPRTPPY
3) YKTIAFDEEARR
6) EALIIQLKINPYVLS
7) QYIKANAKFIGITE
8) Valli et al., J. Clin. Invest. 91:616, 1993.

Table II

An algorithm to predict DRB1*0401 binding capacity.
a) Original peptide set.

Selection Criteria	No. of peptides (Binding nM) High ≤100	Inter. 100-1000	Non >1000	Total
None	27	53	304	384
P1-P6	27	50	182	259
-17.00 [1]	27	45	84	156
-16.44 [2]	25	35	47	107

1) Algorithm score which predicts 90% of all binders.

2) Algorithm score which predicts 75% of all binders.

Table II

b) Blind test of the predictive power of the DRB1*0401 algorithm.

Selection Criteria	No. of peptides (Binding nM) High ≤100	Inter. 100-1000	Non >1000	Total
None	3	11	36	50
P1-P6	3	9	28	40
-17.00	3	8	7	18
-16.44	3	4	2	9

Table III

A combined "1-4-7" algorithm.

Selection Criteria	Degenerate Binders [1]	Percent of Total Degenerate Binders
None	73/384	100%
P1-P6	72/259	99%
Combined Algorithms (90% Cutoff Value)	67/147	92%
Combined Algorithms (75% Cutoff Value)	59/100	81%

1) Degenerate binders are defined as peptides binding at least two out of the three DR1, 4w4, and 7 molecules with an IC50 of 1 μM or less.

Table IV

Phenotypic frequencies of 10 prevalent HLA-DR antigens

Antigen	Alleles	Phenotypic Frequencies					
		Cauc.	Blk.	Jpn.	Chn.	Hisp.	Avg.
DR1	DRB1*0101-03	18.5	8.4	10.7	4.5	10.1	10.4
DR2	DRB1*1501-03	19.9	14.8	30.9	22.0	15.0	20.5
DR3	DRB1*0301-2	17.7	19.5	0.4	7.3	14.4	11.9
DR4	DRB1*0401-12	23.6	6.1	40.4	21.9	29.8	24.4
DR7	DRB1*0701-02	26.2	11.1	1.0	15.0	16.6	14.0
DR8	DRB1*0801-5	5.5	10.9	25.0	10.7	23.3	15.1
DR9	DRB1*09011,09012	3.6	4.7	24.5	19.9	6.7	11.9
DR11	DRB1*1101-05	17.0	18.0	4.9	19.4	18.1	15.5
DR12	DRB1*1201-02	2.8	5.5	13.1	17.6	5.7	8.9
DR13	DRB1*1301-06	21.7	16.5	14.6	12.2	10.5	15.1
Total		97.0	83.9	98.8	95.5	95.6	94.7

Table V

A) Non Predictions.

	Binding Capacity												
	DR1,4,7			Other Alleles									
Peptide	DR1	DR4w4	DR7	DR2w2b	DR2w2a	DR3	DR4w15	DR5w11	DR6w19	DR8w2	DR9	DR5w12	Total Alleles Bound
1136.29	32	4317	138	1.1	468	–	745	6250	–	2970	183	1000	7
1136.04	24	20	3333	1264	741	–	563	69	–	55	2885	–	6
1136.19	781	1915	1323	86	1250	–	445	183	1667	5052	3125	–	4
1136.49	–	–	505	–	702	–	250	645	–	1581	4167	9091	4
1136.02.01a	806	–	–	2844	16	–	–	1379	–	338	–	9927	3
1136.35	116	–	–	2459	–	–	1086	126	8750	306	–	1364	3
1136.52	–	7031	556	3957	1667	–	563	–	–	–	2419	–	2
1136.03	79	8654	2083	243	1250	–	1689	–	–	7313	3947	3571	2
1136.06	1923	1364	–	–	313	6977	–	690	8750	–	–	–	2
1136.23	962	–	–	262	–	2727	–	–	3182	–	–	–	2
1136.32	37	–	–	1717	1739	–	626	6250	–	1776	–	–	2
1136.33	52	–	–	8273	6250	–	7600	1835	8750	3161	–	476	2
1136.44.01	526	780	–	–	–	–	6552	4000	–	6364	–	–	2
1136.42.01a	–	–	–	–	449	–	–	396	–	2970	3000	–	2
1136.42	–	1875	–	–	769	–	–	9524	8750	–	–	–	1
1136.54	8333	–	–	–	–	–	–	–	781	–	–	2727	1
1136.07.01b	1190	–	4630	1542	2857	–	–	1980	–	1225	2614	214	1
1136.05	–	492	–	–	–	–	–	–	–	–	–	–	1
1136.08	–	9375	3788	73	–	–	–	–	–	–	2027	–	1
1136.25	1163	–	6250	28	3846	–	–	–	2917	–	–	3846	1
1136.34	4545	545	3247	–	–	–	–	–	–	–	5000	–	1
1136.36	204	–	–	5688	–	–	–	–	–	–	12931	–	1
1136.64	–	225	–	–	–	–	1267	–	5000	–	–	–	1
1136.69	–	–	–	–	–	–	–	–	54	–	5769	–	1
1136.40	4545	1546	8333	–	4348	–	–	–	7000	–	–	–	0
1136.50	–	1875	–	–	–	–	6667	7143	–	5506	–	–	0
1136.56	–	4500	–	–	–	–	3918	–	3500	–	–	–	0
1136.57	–	8654	–	6500	–	–	5758	1626	–	5104	4688	–	0
1136.61	–	–	–	–	–	–	–	–	–	–	7979	–	0
1136.66	–	–	–	–	–	–	–	–	–	–	–	–	0
1136.68	–	–	–	–	–	–	–	–	–	–	–	–	0
1136.70	–	–	–	–	–	–	–	3704	–	–	–	–	0
1136.72	–	–	–	–	–	–	–	–	–	–	–	–	0
1136.63.01a	–	–	–	–	1905	–	–	7692	–	4298	–	–	0

– indicates binding affinity ≥10,000nM.

2 out of 34 (5.9%) degenerate on 5 or more DR types.

Table V

B) Correct Predictions.

	Binding Capacity (IC50% nM)												
	DR1,4,7			Other Alleles									
Peptide	DR1	DR4w4	DR7	DR2w2b	DR2w2a	DR3	DR4w15	DR5w11	DR6w19	DR8w2	DR9	DR5w12	Total Alleles Bound
1188.16	3.7	7.1	14	1251	23	–	47	30	428	46	28	–	9
1188.32	3.1	44	167	–	29	–	1402	11	7.1	19	126	851	9
1188.34	14	12	66	370	148	1332	959	2703	3.7	68	19	497	9
F107.09	4.1	14	39	5028	286	–	324	963	469	385	29	–	9
27.412	14	282	138	–	323	–	–	31	20	53	590	2495	8
1188.45	26	9.0	57	260	123	757	1057	2532	3.9	28	16	–	8
1136.16	1.6	214	46	1625	34	–	741	3571	1296	488	68	3409	7
1136.21	2.2	51	52	2844	62	–	270	1212	259	1420	132	–	7
1136.11	0.89	99	9615	603	261	–	84	315	–	529	1974	–	7
27.392	41	449	33	310	2499	–	1668	1203	9.8	883	62	–	6
27.417	56	–	425	210	251	–	–	471	33	2177	859	3243	6
1136.38	70	122	2404	258	741	–	133	4000	1842	–	862	–	6
27.388	50	5737	497	18	1536	–	1410	542	38	–	708	2512	5
27.403	78	4146	207	13	2875	–	–	73	66	1672	423	7321	5
1136.71	5.1	776	96	–	1212	–	950	1538	–	–	375	–	5
1136.14	5.3	4787	100	81	135	–	792	–	1400	–	7732	3488	5
1136.24	182	5844	391	506	9574	–	1357	–	6.5	–	–	–	4
27.384	66	–	281	357	–	–	–	–	65	–	458	–	4
1188.13	116	6923	58	382	–	–	1069	–	0.77	–	142	–	4
F107.10	120	2728	67	807	–	–	1647	–	5.5	–	135	–	4
F107.17	221	388	–	–	–	4878	5705	–	76	7640	299	3478	4
F107.23	163	5713	141	4413	–	–	6770	–	14	–	151	–	4
1136.12	105	720	1429	14	2128	–	1583	–	343	2917	2500	–	4
1136.47	2.2	407	2119	303	755	–	5352	4255	–	–	–	–	4
1136.28	0.23	849	3623	2.2	1481	–	6667	9524	3182	7538	–	4478	3
1136.55	65	138	2451	–	–	–	271	4545	5000	–	–	–	3
1134.59.01a	130	39	–	–	29	–	3140	–	–	–	–	–	3
27.415	2011	754	718	653	–	–	–	4712	2234	8997	–	–	2
1136.46	68	985	5814	–	–	–	–	–	–	–	–	–	2

– Indicates binding affinity ≥10,000nM.

16 out of 29 (55%) degenerate on 5 or more DR types.

Table VI

Degenerate "1-4-7" binders.

		Binding Capacity (IC50% nM)											
		DR1,4,7			Other Alleles								
Peptide	Sequence	DR1	DR4w4	DR7	DR2w2b	DR2w2a	DR3	DR4w15	DR5w11	DR6w19	DR8w2	DR9	DR5w12	Total Alleles Bound
1188.34	IINWVNIIAVPLAMKLI	+	+	+	+	+	−	+	−	+	+	+	+	10
1188.32	GLAYKFVVPGAATPY	+	+	+	−	+	−	−	+	+	+	+	+	9
1188.16	KSKYKLATSVLAGLL	+	+	+	−	+	−	+	+	+	+	+	−	9
F107.09	KYKLATSVLAGLLGN	+	+	+	−	+	−	+	+	+	+	+	−	9
1188.45	RIINWVNIIAVPLAMKL	+	+	+	+	+	+	−	−	+	+	+	−	8
27.412	AYKFVVPGAATPYAG	+	+	+	−	+	−	+	+	+	+	−	−	7
1136.11	VVFPASFFIKLPIILA	+	+	−	+	+	−	+	+	−	+	+	−	7
1136.16	LTSQFFLPALPVFTWL	+	+	+	−	+	−	+	−	−	−	+	−	7
1136.21	IPQEWKPAITVKVLPA	+	+	+	−	+	−	+	−	−	−	+	+	7
1136.29	GPPTALRSFGFAFGYM	+	−	+	+	+	−	+	−	+	−	+	−	7
27.392	SSVFNVVNSSIGLIM	+	+	+	+	−	−	−	+	+	−	+	−	7
27.417	VKNVIGPFMKAVCVE	+	−	+	−	+	−	−	+	+	−	−	−	6
1136.04	LFIIYYFLSEKAPGSTV	+	+	−	+	−	−	−	+	+	−	+	−	6
27.388	MRKLAILSVSSFLFV	+	−	+	+	+	−	−	−	+	−	+	−	6
1136.38	SSIIFGAFTSLIISGCC	+	+	−	+	−	−	−	+	−	−	+	−	5
27.403	LVNLLIFIIINGKIIK	+	−	+	−	−	−	−	+	−	−	−	−	5
1136.71	EPQGSTYAASSATSVD	+	+	+	+	−	−	−	+	−	−	+	−	5
1136.14	FATCFLIPLTSQFFLP	+	−	+	+	−	−	−	−	+	−	+	−	5
27.384	FNVVNSSIGLIMVIS	+	−	+	+	−	−	−	−	+	−	+	−	5
1188.13	AGLLGNVSTVLLGGV	+	−	+	+	−	−	−	−	+	−	−	−	4
F107.10	LAGLLGNVSTVLLGG	+	+	−	+	+	−	−	−	−	−	−	−	4
1136.47	TIIIIYFVDLIGGAMLSL	+	+	−	+	−	−	−	−	+	−	+	−	4
1136.12	IKLPIILAFATCFLIP	+	−	+	−	−	−	−	−	+	−	−	−	4
F107.23	VFNVVNSSIGLIMVL	+	−	+	+	−	−	−	−	+	−	+	−	4
1136.24	NLSNVLATIITGVLDI	+	+	−	−	−	−	−	−	−	−	−	−	3
F107.17	KFVVPGAATPYAGEP	+	+	−	+	−	−	−	−	−	−	−	−	3
1136.28	LAAIIFLFGPPTALRS	+	+	−	−	−	−	+	−	−	−	−	−	3
1136.55	QEIDPLSYNYIPVNSN	+	+	−	−	+	−	−	−	−	−	−	−	3
1136.59.01a	RVYQEPQVSTPQRAET	+	+	−	+	−	−	−	−	−	−	−	−	2
27.415	NVKYLVIVFLIFFDL	−	+	+	−	−	−	−	−	−	−	−	−	2
1136.46	LWWSTMYLTIIIIYFVDL	+	+	−	−	−	−	−	−	−	−	−	−	2
1136.44.01	WLFPRFKFVWVTYASW	+	+	−	−	−	−	−	−	−	−	−	−	2
		31	22	21	18	16	1	12	9	18	10	20	3	

+ indicates binding affinity ≤1000nM.

Table VII

DR Type	Frequency of Binders	
	1-4-7 Degenerate Binders (%)	Non 1-4-7 Degenerate Binders (%)
1	31/32 (97)	17/67 (25)
4w4	22/32 (69)	8/67 (12)
7	21/32 (66)	7/67 (10)
9	20/32 (62)	2/67 (3.0)
6w19	18/32 (56)	6/67 (8.9)
2w2β b	18/32 (56)	16/67 (24)
2w2β a	16/32 (50)	10/67 (15)
4w15	12/32 (37)	4/67 (6.0)
8w2	10/32 (31)	3/67 (4.5)
5w11	9/32 (28)	6/67 (8.9)
5w12	3/32 (9.4)	4/67 (6.0)
3w17	1/32 (3.1)	0/67 (0)
w53	2/16 (13)	7/43 (16)

Table VIII, page 1

In IC50 Format

Peptide	Sequence	Source	DR1 nM	DR2w2β1 nM	DR2w2β2 nM	DR3 nM	DR4w4 nM	DR4w14 nM	DR4w15 nM	DR5w11 nM	DR5w12 nM	DR6w19 nM	DR7 nM	DR8w2 nM	DR9 nM	DRw53 nM
27.0411	VNLLFHNGIGIGI	Pf LSA1 14	13.9	8.1	181.8	2307.7	8429.8		4835.1000	222.2	8081	30.3	29.8	538.4	138.0	
39.0217	RWCIPWQRLLLTASL	CEA 10	0.2	827.3	(28571.4)		542.2			(40000.0)		318.2	357.1	(122500.0)		
39.0218	QRLLLTASLLTFWNP	CEA 16	(12500.0)				(20454.5)						(18230.8)			
39.0219	EVLLLVHNLPQHLFG	CEA 50	2.0	38.8	15384.6		52.3			858.2		1.0	53.2	408.3		
39.0220	GREIIYPNASLLIQN	CEA 97	8.1	23.0	8333.3		483.8			6896.6		2.8	44.8	5903.6		
39.0221	EIIYPNASLLIQNII	CEA 99	14.3	58.8	(28571.4)		1153.8			(40000.0)		11.3	158.3	16896.6		
39.0222	NASLLIQNIIQNDTG	CEA 104	4545.5				(20454.5)						(18230.8)			
39.0223	DTGFYTLHVIKSDLV	CEA 116	69.4	505.6	800.0		1730.8			2500.0		3846.9	227.3	780.3		
39.0224	YPELPKPSISSNNSK	CEA 141	5555.6				(20454.5)						(18230.8)			
39.0225	KPSISSNNSNPVEDK	CEA 146	2301.0				(20454.5)						7575.8			
39.0226	YLWWVNNQSLPVSPR	CEA 176	6.6	108.8	2105.3		8.0			28.6		2.3	41.7	1085.2		
39.0227	LWWVNNQSLPVSPRL	CEA 177	217.4				1551.7						3048.8			
39.0228	QYSWFVNGTFQQSTQ	CEA 288	192.3	(13000.0)	6060.6		80.4			370.4		5833.3	925.9	12584.1		
39.0229	DTGLNRTTVTTITVY	CEA 305	(12500.0)				(20454.5)						2640.8			
39.0230	KPFITSNNSNPVEDE	CEA 324	(12500.0)				(20454.5)						(18230.8)			
39.0231	NRTLTLLSVTRNDVG	CEA 375	238.1				(20454.5)						11904.8			
39.0232	QELFISNITEKNSGL	CEA 460	10000.0				2500.0						(18230.8)			
39.0233	RTTVKTITVSAELPK	CEA 488	454.5	364.0	22222.2		7031.3			(40000.0)		700.0	318.5	(122500.0)		
39.0234	SAELPKPSISSNNSK	CEA 497	(12500.0)				(20454.5)						(18230.8)			
39.0235	LDVLYGPDTPIISPP	CEA 567	(12500.0)				(20454.5)						(18230.8)			
39.0236	TQVLFIAKITPNNNG	CEA 637	61.0				12162.2						8578.9			
39.0237	QVLFIAKITPNNNGT	CEA 638	41.7				1875.0						10418.7			
39.0238	YACFVSNLATGRNNS	CEA 653	208.3				1888.7						3571.4			
39.0239	NNSIVKSITVSASGT	CEA 665	80.9	3137.9	(28571.4)		25.0			12500.0		50.7	873.7	4083.3		
39.0240	NSIVKSITVSASGTS	CEA 666	78.1	3858.5	(28571.4)		25.0			16666.7		78.1	328.9	2882.4		
39.0241	LCRWGLLLALLPPGA	Her2/neu.6	53.2				(18000.0)						11904.8			
39.0242	RWGLLLALLPPGAAS	Her2/neu.8	0.4	70.0	740.7		160.7			281.7		(11888.7)	(18230.8)	408.3		
39.0243	WGLLLALLPPGAAST	Her2/neu.9	1.0	43.3	1818.2		34.6			80.0		(11888.7)	(18230.8)	108.9		
39.0244	GTDMKLRLPASPETH	Her2/neu.28	8000.0				(18000.0)						(18230.8)			
39.0245	DMKLRLPASPETHLD	Her2/neu.30	5000.0				(18000.0)						(18230.8)			
39.0246	NLELTYLPTNASLSF	Her2/neu.59	10.8	325.0	2222.2		118.4			4000.0		2058.8	387.6	2227.3		
39.0247	LTYLPTNASLSFLQD	Her2/neu.62	10.2	810.0	357.1		138.4			4878.0		125.0	78.1	8074.1		
39.0248	TQLFEDNYALAVLDN	Her2/neu.105	94.3				(18000.0)						1562.5			
39.0249	VCPLHNQEVTAEDGT	Her2/neu.314	(12500.0)				(18000.0)						(18230.8)			
39.0250	CKKIFGSLAFLPESF	Her2/neu.367	20.8	1300.0	12500.0		(18000.0)			(40000.0)		1029.4	925.9	(122500.0)		
39.0251	LSVFQNLQVIRGRIL	Her2/neu.422	27.8	325.0	270.3		871.6			2000.0		614.0	88.2	1484.8		
39.0252	LRELGSGLALIHHNT	Her2/neu.458	151.5				(18000.0)						(18230.8)			
39.0253	KPDLSYMPIWKFPDE	Her2/neu.605	151.5				(18000.0)						8620.7			
39.0254	ASPLTSIISAVVGIL	Her2/neu.648	84.2	95.8	9405.4		(18000.0)			(40000.0)		72.0	714.3	(122500.0)		
39.0255	LTSIISAVVGILLVV	Her2/neu.651	29.3				(18000.0)						6102.0			
39.0256	VVGILLVVVLGVVFG	Her2/neu.658	(12500.0)				(18000.0)						(18230.8)			
39.0257	LLVVVLGVVFGILIK	Her2/neu.662	(8250.0)				(18000.0)						13157.9			
39.0258	VLGVVFGILIKRRQQ	Her2/neu.666	71.4	827.3	322.6		13517.2			42.8		333.3	781.3	78.8		
39.0259	ETELVEPLTPSGAMP	Her2/neu.683	833.3				(18000.0)						(18230.8)			
39.0260	VEPLTPSGAMPNQAQ	Her2/neu.687	(8250.0)				(18000.0)						(22727.3)			
39.0261	ETELRKVKVLGSGAF	Her2/neu.717	312.5	4783.5	3846.2		1285.7			3278.7		2500.0	657.9	1000.0		
39.0262	GENVKIPVAIKVLRE	Her2/neu.743	79.4	1836.2	6482.4		(14062.5)			(40000.0)		8750.0	806.5	49000.0		
39.0263	IKVLRENTSPKANKE	Her2/neu.752	(10000.0)				(14062.5)						(22727.3)			
39.0264	KEILDEAYVMAGVGS	Her2/neu.765	1388.9				6184.4						(22727.3)			
39.0265	DEAYVMAGVGSPYVS	Her2/neu.768	100.0	3137.9	833.3		193.7			7407.4		1750.0	125.0	858.6		
39.0266	SRLLGICLTSTVQLV	Her2/neu.782	14.3	413.6	(28571.4)		375.0			1428.6		10.3	44.6	54444.4		
39.0267	TVQLVTQLMPYGCLL	Her2/neu.793	21.7	12.1	22222.2		878.3			(40000.0)		1129.0	2500.0	7101.4		
39.0268	LLNWCMQIAKGMSYL	Her2/neu.822	8.0	1398.5	16.7		40909.1			60.0		89.7	208.3	118.5		
39.0269	ITDFGLARLLDIDET	Her2/neu.861	1041.7				(14062.5)						(22727.3)			
39.0270	KVPIKWMALESILRR	Her2/neu.883	2.3	2.4	9.5		852.2			2739.7		1129.0	1315.8	8202.5		
39.0271	PIKWMALESILRRRF	Her2/neu.885	6.3				1285.7						3205.1			
39.0272	IKWMALESILRRRFT	Her2/neu.886	6.3				1125.0						6250.0			
39.0273	GVTVWELMTFGAKPY	Her2/neu.909	3.6				1383.6						1470.6			
39.0274	VWELMTFGAKPYDGI	Her2/neu.912	57.5	81.9	200.0		818.2			3703.7		8750.0	873.7	5505.8		

Table VIII, page 2

In IC50 Format

Peptide	Sequence	Source	DR1 nM	DR2w2β1 nM	DR2w2β2 nM	DR3 nM	DR4w4 nM	DR4w14 nM	DR4w10 nM	DR5w11 nM	DR5w12 nM	DR6w19 nM	DR7 nM	DR8w2 nM	DR9 nM	DRw53 nM
39.0275	GERLPQPPICTIDVY	Her2/neu.936	(10000.0)				(14062.5)						(22727.3)			
39.0276	QPPICTIDVYMIMVK	Her2/neu.943	74.6	108.5	7407.4		7500.0			4347.8		2632.3	250.0	8807.8		
39.0277	DVYMIMVKCWMIDSE	Her2/neu.950	178.6	1838.2	4761.9		789.5			909.1		(11666.7)	192.3	1060.9		
39.0278	QGFFCPDPAPGAGGM	Her2/neu.1028	(10000.0)				1958.5						(22727.3)			
39.0279	TDGYVAPLTCSPQPE	Her2/neu.1124	(10000.0)				(14062.5)						(22727.3)			
39.0280	QPDVRPQPPSPREGP	Her2/neu.1142	7142.9				(14062.5)						(22727.3)			
39.0281	PSTFKGTPTAENPEY	Her2/neu.1234	(10000.0)				(14062.5)						(22727.3)			
39.0282	LGEVPAAESPDPPQS	MAGE2.50	(10000.0)				(14062.5)						(22727.3)			
39.0283	ESEFQAAISRKMVEL	MAGE2.102	4.2	148.8	20.8		281.3			740.7		522.4	49.0	1580.6		
39.0284	GIEVVEVVPISHLYI	MAGE2.163	595.2	1976.3	22222.2		8428.6			(40000.0)		49.3	277.8	6503.8		
39.0285	DGLLGDNQVMPKTGL	MAGE2.187	(10000.0)				(14062.5)						(22727.3)			
39.0286	NQVMPKTGLLIIVLA	MAGE2.193	2631.6				(14062.5)						(22727.3)			
39.0287	KTGLLIIVLAIIAIE	MAGE2.196	418.7	2459.5	(8890.0)		1216.2			(40000.0)		2333.3	862.1	(122500.0)		
39.0288	TGLLIIVLAIIAIEG	MAGE2.199	6250.0				(14062.5)						(22727.3)			
39.0291	GLLIIVLAIIAIEGD	MAGE2.200	500.0				(14062.5)						(22727.3)			
39.0292	LLIIVLAIIAIEGDC	MAGE2.201	381.4				3750.0						1923.1			
39.0293	LIIVLAIIAIEGDCA	MAGE2.202	416.7				6823.5						2083.3			
39.0294	EPHISYPPLHERALR	MAGE2.296	(16666.7)				(14062.5)						(22727.3)			
39.0295	ALGLVGAQAPATEEQ	MAGE2/3.22	151.5				(14062.5)						(22727.3)			
39.0296	ESEFQAALSRKVAEL	MAGE3.102	2.8	29.4	16.5		783.7			645.2		7000.0	33.6	1139.5		
39.0297	NWQYFFPVIFSKASS	MAGE3.142	45.5	3033.3	606.7		409.1			307.7		(11666.7)	444.4	222.7		
39.0298	PVIFSKASSSLQLVF	MAGE3.148	90.0	525.3	(28571.4)		1875.0			(40000.0)		145.8	290.8	13894.7		
39.0299	LQLVFGIELMEVDPI	MAGE3.158	200.0	4550.0	15384.6		(14062.5)			(40000.0)		8750.0	257.7	(122500.0)		
39.0300	GHLYIFATCLGLSYD	MAGE3.173	454.5				4090.9						10000.0			
39.0301	DGLLGDNQIMPKAGL	MAGE3.187	(16666.7)				(14062.5)						(22727.3)			
39.0302	NQIMPKAGLLIIVLA	MAGE3.193	112.0				(14062.5)						(22727.3)			
39.0303	KAGLLIIVLAIIARE	MAGE3.196	1162.8				(50250.0)						(22727.3)			
39.0304	AGLLIIVLAIIAREG	MAGE3.199	1111.1				(50250.0)						(9615.4)			
39.0305	LLIIVLAIIAREGDC	MAGE3.201	1923.1				(50250.0)						(13888.9)			
39.0306	GPHISYPPLHEWVLR	MAGE3.296	2272.7				(16666.7)						(13888.9)			
39.0307	GFRLGFLHSGTAKSV	p53.108	2.6	252.0	166.7		8.4			100.0		76.1	90.3	29.8		
39.0308	LNKMFCQLAKTCPVQ	p53.130	20.0	5687.5	540.5		803.6			2500.0		364.0	166.7	1531.3		
39.0309	MGGMNRRPILTIITL	p53.243	(10000.0)				(16666.7)						(35714.3)			
39.0310	RRPILTIITLEDSSG	p53.248	5000.0				4500.0						10869.6			
39.0311	KRALPNNTSSSPQPK	p53.305	(10000.0)				(16666.7)						(35714.3)			
39.0312	DGEYFTLQIRGRERF	p53.324	125.0				(16666.7)						(13888.9)			
39.0313	QNIIQNDTGFYTLHV	CEA.110	1136.4	866.7	(28571.4)	937.5				(25000.0)		8.7	(14705.9)	(54444.4)		
39.0314	LHVIKSDLVNEEATG	CEA.122				2307.7										
39.0315	KSDLVNEEATGQFRV	CEA.126				51724.1										
39.0316	SDLVNEEATGQFRVY	CEA.127				(111111.1)										
39.0317	NEEATGQFRVYPELP	CEA.131				(111111.1)										
39.0318	QFRVYPELPKPSISS	CEA.137				(111111.1)										
39.0319	AVAFTCEPETQDATY	CEA.162				(111111.1)										
39.0320	TASYKCETQNPVSAR	CEA.210				(111111.1)										
39.0321	NVLYGPDAPTISPLN	CEA.232				(111111.1)										
39.0322	ISPLNTSYRSGENLN	CEA.242				(111111.1)										
39.0323	SGSYTCQAHNSDTGL	CEA.294				(111111.1)										
39.0324	TITVYAEPPKPFITS	CEA.315				71428.6										
39.0325	SNPVEDEDAVALTCE	CEA.332				55555.6										
39.0326	AVALTCEPEIQNTTY	CEA.340				76923.1										
39.0327	NQSLPVSPRLQLSND	CEA.360				42253.5										
39.0328	RLQLSNDNRTLTLLS	CEA.366	50000.0	(15166.7)	(28571.4)	937.5				(25000.0)		729.2	(14705.9)	(54444.4)		
39.0329	ECGIQNELSVDHSDP	CEA.392				(111111.1)										
39.0330	QNELSVDHSDPVILN	CEA.396				3656.5										
39.0331	NVLYGPDDPTISPSY	CEA.410				(111111.1)										
39.0332	GVNLSLSCHAASNPP	CEA.430				40000.0										
39.0333	TITVSAELPKPSISS	CEA.493				(111111.1)										
39.0334	AVAFTCEPEAQNTTY	CEA.518				(111111.1)										
39.0335	SDPVTLDVLYGPDTP	CEA.582				(111111.1)										

Table VIII, page 3

In IC50 Format

Peptide	Sequence	Source	DR1 nM	DR2w2β1 nM	DR2w2β2 nM	DR3 nM	DR4w4 nM	DR4w14 nM	DR4w15 nM	DR5w11 nM	DR5w12 nM	DR6w19 nM	DR7 nM	DR8w2 nM	DR9 nM	DRw53 nM
39.0336	DALYGPDTPIISPPD	CEA.586				81081.1										
39.0337	GANLNLSCHSASNPS	CEA.605				(111111.1)										
39.0338	RLPASPETHLDMLRH	Her2/neu.34				(111111.1)										
39.0339	SLSFLQDIQEVQGYV	Her2/neu.70				5768.2										
39.0340	VLIAHNQVRQVPLQR	Her2/neu.84				10344.8										
39.0341	GTQLFEDNYALAVLD	Her2/neu.104				1363.6										
39.0342	DTILWKDIFHKNNQL	Her2/neu.185				(111111.1)										
39.0343	ALTLIDTNRSRACHP	Her2/neu.190				8571.4										
39.0344	KGPLPTDCCHEQCAA	Her2/neu.228				(111111.1)										
39.0345	LVTYNTDTFESMPNP	Her2/neu.271				(111111.1)										
39.0346	YNYLSTDVGSCTLVC	Her2/neu.301				30847.8										
39.0347	NQEVTAEDGTQRCEK	Her2/neu.319				(111111.1)										
39.0348	CYGLGMEHLREVRAV	Her2/neu.342				38144.0										
39.0349	SLAFLPESFDGDPAS	Her2/neu.373				(111111.1)										
39.0350	PESFDGDPASNTAPL	Her2/neu.379				(111111.1)										
39.0351	TAPLQPEQLQVFETL	Her2/neu.389				130434.8										
39.0352	LALIHHNTHLCFVHT	Her2/neu.465	337.1	91.0	22222.2	967.7				8000.0		4.7	1250.0	1464.8		
39.0353	VHTVPWDQLFRNPHQ	Her2/neu.477				13636.4										
39.0354	WDQLFRNPHQALLHT	Her2/neu.482	(50000.0)	9088.7	(28571.4)	333.3				(25000.0)		85.4	(14705.9)	(94444.4)		
39.0355	LQGLPREYVNARHCL	Her2/neu.547				(111111.1)										
39.0356	VTCFGPEADQCVACA	Her2/neu.574				(111111.1)										
39.0357	PSGVKPDLSYMPIWK	Her2/neu.601				(111111.1)										
39.0358	IWKFPDEEGACQPCP	Her2/neu.613				(111111.1)										
39.0359	HSCVDLDDKGCPAEQ	Her2/neu.632				(111111.1)										
39.0360	MRRLLQETELVEPLT	Her2/neu.667				(111111.1)										
39.0361	QMRILKETELRKVKV	Her2/neu.711	118.3	908.7	83.8	937.5				4878.0		4375.0	1822.1	7858.3		
39.0362	AIKVLRENTSPKANK	Her2/neu.751				40000.0										
39.0363	NKEILDEAYVMAGVG	Her2/neu.764				63829.8										
39.0364	GMSYLEDVRLVHRDL	Her2/neu.832				1886.7										
39.0365	VRLVHRDLAARNVLV	Her2/neu.839	147.1	1421.9	8060.6	982.4				74.1		81.4	1087.0	490.0		
39.0366	ARLLDIDETEYHADG	Her2/neu.867	50000.0	(15166.7)	(28571.4)	987.7				(25000.0)		(35000.0)	(14705.9)	(94444.4)		
39.0367	ETEYHADGGKVPIKW	Her2/neu.874				(111111.1)										
39.0368	IKWMALESILRRRFT	Her2/neu.888	18.7	10.8	2.5	841.8				370.4		2500.0	4088.4	731.3		
39.0369	CWMIDSECRPRFREL	Her2/neu.958	[illegible]	(15166.7)	1333.3	866.7				(25000.0)		(35000.0)	(17857.1)	17500.0		
39.0370	FRELVSEFSRMARDP	Her2/neu.969				4225.4										
39.0371	FSRMARDPQRFVVIQ	Her2/neu.978				1875.0										
39.0372	FVVIQNEDLGPASPL	Her2/neu.988				(120000.0)										
39.0373	YRSLLEDDDMGDLVD	Her2/neu.1005				4761.9										
39.0374	RSLLEDDDMGDLVDA	Her2/neu.1006				37500.0										
39.0375	GDLVDAEEYLVPQQG	Her2/neu.1015				12000.0										
39.0376	GFFCPDPAPGAGGMV	Her2/neu.1026				66666.7										
39.0377	DLTLGLEPSEEEAPR	Her2/neu.1058				(120000.0)										
39.0378	SDVFDGDLGMGAAKG	Her2/neu.1083				(120000.0)										
39.0379	LQRYSEDPTVPLPSE	Her2/neu.1109				11111.1										
39.0380	TVPLPSETDGYVAPL	Her2/neu.1117				(120000.0)										
39.0381	KNGVVKDVFAFGGAV	Her2/neu.1177				(120000.0)										
39.0382	GGAAPQPHPPPAFSP	Her2/neu.1200				(120000.0)										
39.0383	DNLYYWDQDPPERGA	Her2/neu.1218				(120000.0)										
39.0384	QPRMFPDLESEFQAA	MAGE2.94				3370.8										
39.0387	FPDLESEFQAAISRK	MAGE2.98				90909.1										
39.0388	EFQAAISRKMVELVH	MAGE2.104				76923.1										
39.0389	QLVFGIEVVEVVPIS	MAGE2.159				41666.7										
39.0390	CLGLSYDGLLGDNQV	MAGE2.181				2142.9										
39.0391	YDGLLGDNQIMPKTG	MAGE2.186				11111.1										
39.0392	LAAIEGDCAPEEK	MAGE2.206				15789.5										
39.0393	IAIEGDCAPEEKIW	MAGE2.208				4545.5										
39.0394	EEKIWEELSMLEVFE	MAGE2.218				23076.9										
39.0395	RKLLMQDLVQENYLE	MAGE2.243				2000.0										
39.0396	MQLVQLNYRFRQVPD	MAGE2.247				1500.0										

Table VIII, page 4

In IC50 Format

Peptide	Sequence	Source	DR1 nM	DR2w2β1 nM	DR2w2β2 nM	DR3 nM	DR4w4 nM	DR4w14 nM	DR4w15 nM	DR5w11 nM	DR5w12 nM	DR6w19 nM	DR7 nM	DR8w2 nM	DR9 nM	DRw53 nM
39.0387	VKVLHHTLKIGGEPH	MAGE2.284				(120000.0)										
39.0388	TLKIGGEPHISYPPL	MAGE2.290				(120000.0)										
39.0399	FPDLESEFQAALSRK	MAGE3.98				115384.6										
39.0400	EFQAALSRKVAELVH	MAGE3.104				111111.1										
39.0401	DLVFGIELMEVDPIG	MAGE3.119				20000.0										
39.0402	ELMEVDPIGHLYIF	MAGE3.124	18666.7	1386.8	(20000.0)	184.7				(25000.0)		259.2	9256.3	3789.2		
39.0403	DLGLSYDGLLGDNQI	MAGE3.181				(120000.0)										
39.0404	YDGLLGDNQIMPKAG	MAGE3.186				37500.0										
39.0405	LAIIAREGDCAPEEK	MAGE3.209				(120600.0)										
39.0406	EEKIWEELSVLEVFE	MAGE3.219				51724.1										
39.0407	EDSILGDPKKLLTQH	MAGE3.235	18666.7	(15188.7)	(20000.0)	447.8				(25000.0)		281.2	(17857.1)	15896.6		
39.0408	TQHFVQENYLEYRQV	MAGE3.247				8071.4										
39.0409	EPPLSQETFSDLWKL	p53.11				(120000.0)										
39.0410	LWKLLPENNVLSPLP	p53.22				103448.3										
39.0411	DLMLSPDDIEQWFTE	p53.42				20000.0										
39.0412	EQWFTEDPGPDEAPR	p53.51				(120000.0)										
39.0413	PVQLWVDSTPPPGTR	p53.142				10000.0										
39.0414	MAIYKQSQHMTEVVR	p53.160				(120000.0)										
39.0415	QHLIRVEGNLRVEYL	p53.182				3125.0										
39.0416	LIRVEGNLRVEYLDD	p53.184				3225.8										
39.0417	EGNLRVEYLDDRNTF	p53.188				75823.1										
39.0418	RVEYLDDRNTFRHSV	p53.202				1600.7										
39.0419	SVVVPYEPPEVGSDC	p53.215				(120000.0)										
39.0420	PPEVGSDCTTIHYNY	p53.222				7894.7										
39.0421	LTIITLEDSSGNLLG	p53.252				100000.0										
39.0422	KKPLDGEYFTLQIRG	p53.320				(120000.0)										
39.0423	GEYFTLQIRGRERFE	p53.325				10344.8										
39.0424	RFEMFRELNEALELK	p53.337				57692.3										
597.02	GRTQDENPVVHFFKNIVTPR	Human MBP 78-101/	17.2	9.1	250.0	60000.0	9.8	9.1		133.3			1000.0			
598.01	KYLEFISEAIIHVLHSR	SW Myo 102-118	294.1		20000.0	25000.0	136.4	2590.0		4000.0			337.8			
515.01	PKYVKQNTLKLAT	HA 307-319	5.0	11375.0	45.5	300000.0	45.0	25000.0		117.6			352.1			432.9
515.02	HKYVKQNTLKLAT	HA 307-319 analog	3.8		15.4		21.4	25000.0		180.2			165.6			
515.03	PHYVKQNTLKLAT	HA 307-319 analog	5.0		30.8		28.1	50000.0		243.9			510.2			
515.05	PKYIKQNTLKLAT	HA 307-319 analog	6.3		141.8		40.8	10080.0		512.8			553.8			
515.06	PKYVHQNTLKLAT	HA 307-319 analog	2.8		99.0		18.0	12500.0		444.4			403.2			
515.07	PKYVKHNTLKLAT	HA 307-319 analog	15.7		263.2		60.3	50000.0		290.5			134.4			
515.08	PKYVKQHTLKLAT	HA 307-319 analog	3.8		30.8		37.5	25000.0		370.4			490.2			
515.10	PKYVKQNTHKLAT	HA 307-319 analog	6.3		15.4		178.5	500000.0		4008.0			2777.8			
515.11	PKYVKQNTLHLAT	HA 307-319 analog	5.2		61.2		40.9	25000.0		217.4			500.0			
515.12	PKYVKQNTLKHAT	HA 307-319 analog	434.5		14.8		34.6	3571.4		100.5			4166.7			
515.13	PKYVKQNTLKLHT	HA 307-319 analog	8.7		22.7		60.9	50000.0		840.5			500.0			
515.14	PKYVKQNTLKLAH	HA 307-319 analog	4.5		76.9		40.9	50000.0		350.8			520.8			
519.00	DVFKELKVHHANENI	Ova 15-29	8.8		571.4	150000.0	40.8	250.0		117.6			443.0			
520.01	KIAKMEKASSVFNVVNS	CS 382-398	35.7		3333.3	130434.8	187.5	1282.1		64.5			(1250.0)			
520.02	DIEKKIAKMEKASSVFNVV	CS 378-398	17.2	835.3	1428.6	48153.8	250.0	2272.7		153.8			227.3			58000.0
541.14	IGMYPNLNTHCYKLEH	Bee Venom 79-94	714.2		102.8		2142.8	3571.4		250.0			284.6			
553.01	QYIKANSKFIGITE	TT 830-843	22.7	22758.6	29.0	3290.9	8033.7	250000.0	1481.5000	20.0	30210	4.4	25.0	48.0	76.9	17375.6
553.02	qYIKANSKFIGITEA	TT 830-843 analog	236.1		25.3	103448.3	12162.2	500000.0	745.1000	63.3			40.9	49.0		38966.7
553.23	KSSQYIKANSSFIGITE	KSS-TT 830-843 analog	113.8	47.9	526.3		45.9	294.1	1055.6000	1333.3			131.6	10652.2		1705.9
553.24	KSSQYIKANSAFIGITE	KSS-TT 830-843 analog	84.2	237.9	181.8		88.2	200.0	750.0000	1052.6			252.5	14000.0		852.8
553.25	KSSQYIKANSLFIGITE	KSS-TT 830-843 analog	138.8	8100.0	476.2		112.5	3848.2	1085.7000	833.3			51.0	25789.5		414.3
553.26	KSSQYIKANSSFIGKTE	KSS-TT 830-843 analog	200.9	23.3	416.7		40.9	200.6	1461.5000	740.7			328.9	18846.2		4481.5
553.27	KSSaQYIKANSSFIGITE	KSS-TT 830-843 analog	131.8	60.7	571.4		60.0	186.7	703.7000	1538.5			106.7	6032.8		39666.7
553.28	KSSQXXKANSSFIGITE	KSS-TT 830-843 analog	102.9	82.7	370.4		93.8	178.8	658.8000	714.3			138.9	25789.5		6079.4
553.29	KSSaQXIKANSSFIGKTE	KSS-TT 830-843 analog	90.3	90.9	229.0		21.4	113.6	745.1000	833.3			30.5	17500.0		4142.9
553.30	KSSaQXIKANSSFIGKTE	KSS-TT 830-843 analog	75.8	10.6	82.5		16.7	122.8	283.3000	266.7			60.3	17500.0		1234.0
553.31	KSSaQXVKANSSFIGKTE	KSS-TT 830-843 analog	72.8	50.9	21.8		51.3	83.3	198.3000	862.4			80.6	2882.4		8800.0
553.32	KSSaQXVKANSSFIGITE	KSS-TT 830-843 analog	20.0	45.0	111.1		14.8	263.2	183.2000	952.4			76.1	5444.4		6041.7
681.01	yPKYVKQNTLKLAr	d protect HA 307-319	1.3		0.1	3000000.0	14.5	6555.6		111.1			73.5			

Table VIII, page 5

In IC50 Format

Peptide	Sequence	Source	DR1 nM	DR2w2β1 nM	DR2w2β2 nM	DR3 nM	DR4w4 nM	DR4w14 nM	DR4w15 nM	DR5w11 nM	DR5w12 nM	DR6w19 nM	DR7 nM	DR8w2 nM	DR9 nM	DRw53 nM
564.02	YVKDAYVKDAYVKDAYVK	Human Reiterative	38.7		18.7		486.8	558.2		8000.0			1823.1			
564.03	YVKQNYVKQNYVKQNYVK	Human Reiterative	1000.0		145.2	75000.0	382.0	500000.0		25000.0			2777.8			
568.00	YYVRAVRAYLTT	Super Ova 323-339	8.5		40.0	3000000.0	7500.0	294.1		215.1			8.3			
570.30	AVLEDPYLLVSSKVS	MT 211-226	11.7		2500.0	100000.0	1022.7	537.6		6666.7			73.3			
571.01	YEHRVKKQLTVAVAGA	19K MT 1-15	20.8	413.8	(6666.7)	150000.0	1000.0	205.8		215.1			(1315.8)			
573.06	PKYVKQNTLKLATGMR	HA 307-322	2.3		44.1		60.8			85.4			97.3			
573.07	YVKQNTLKLATGMR	HA 309-322	7.7		555.8		88.2			487.8			2272.7			
573.10	ILMQYIKANSKFIGITELKK	Tet. Tox. 827-846	10.2		47.8		576.9			25.6			380.6			
573.11	QYIKANSKFIGITELKK	Tet. Tox. 830-846	88.9		13.3		7500.0	50000.0		11.6			25.0			
573.13	ILMQYIKANSKFIGITE	Tet. Tox. 827-843	41.7		27.0		4500.0			14.3			378.6			
573.16	ILMQYIKANSKFIGI	Tet. Tox. 827-841	8.7		23.0		857.4			33.8			382.3			
573.17	ILMQYIKANSKFI	Tet. Tox. 827-839	48.7		11.1		284.7			117.8			104.8			
573.18	ILMQYIKANSK	Tet. Tox. 827-837	500.0		85.2		1666.7			344.8			1000.0			
583.01	FNNFTVSFWLRVPKVSASH	TT 947-969	418.7		2857.1	3000000.0	523.3	4845.5		8.3			58.0			
594.05	YPKFVKQNTLKLAT	HA Y307-319 F/A	3.1		18.2	13043.5	32.1	200.0		66.7			227.3			129.3
597.01	GACPKYVKQNTLKLATGM	HA 304-322	21.7		17.6		34.6			22.2			87.4			
597.02	GACPKYVKQNTLKLAT	HA 304-319	17.9		48.3		54.2			28.7			85.6			
597.03	GACPKYVKQNTLKLA	HA 304-318	12.8		95.7		37.5			129.0			55.2			
597.06	KYVKQNTLKLATGMR	HA 308-322	7.7		204.1		25.0			229.9			78.9			
597.07	YIKANSKFIGITE	Tet. Tox. 831-843	125.0		(153.8)					19.4			25.0			
597.08	ILMQYIKANSKFIG	Tet. Tox. 827-840	108.8		16.7		737.7			2857.1			323.8			
597.09	ILMQYIKANSKF	Tet. Tox. 827-838	833.3		18.2		204.5			1538.5			(1130.4)			
601.01	PKYVKQNTLKLAT	HA 307-319 analog	2.8		77.5		40.8	25000.0		84.0			471.7			
601.02	AKYVKQNTLKLAT	HA 307-319 analog	3.8		18.7		25.0	12500.0		42.0			131.9			
601.03	KKYVKQNTLKLAT	HA 307-319 analog	13.2		40.7		18.8	12500.0		73.5			342.5			
601.04	EKYVKQNTLKLAT	HA 307-319 analog	7.6		55.4		32.1	50000.0		81.8			448.4			
601.05	PRYVKQNTLKLAT	HA 307-319 analog	2.6		44.3		28.1	25000.0		61.2			271.7			
601.06	PEYVKQNTLKLAT	HA 307-319 analog	3.8		61.3		12.5	16666.7		69.2			367.8			
601.07	PSYVKQNTLKLAT	HA 307-319 analog	2.4		37.5		28.1	25000.0		84.0			520.8			
601.08	PKFVKQNTLKLAT	HA 307-319 analog	1.8		83.4		10.0	138.9		42.0			125.8			
601.11	PKYLKQNTLKLAT	HA 307-319 analog	5.7		95.7		60.8	100000.0		165.3			423.7			
601.12	PKYKKQNTLKLAT	HA 307-319 analog	12.8		84.0		108.8	100000.0		78.4			423.7			
601.13	PKYQKQNTLKLAT	HA 307-319 analog	7.7		131.8		34.8	100000.0		180.2			520.8			
601.14	PKYVRQNTLKLAT	HA 307-319 analog	2.0		80.0		25.0	12500.0		69.2			373.1			
601.15	PKYVEQNTLKLAT	HA 307-319 analog	7.4		487.5		48.8	500000.0		1176.5			1785.7			
601.16	PKYVSQNTLKLAT	HA 307-319 analog	2.8		84.1		21.4	10000.0		69.2			357.1			
601.17	PKYVKKNTLKLAT	HA 307-319 analog	13.2		571.4		125.0	25000.0		38.8			189.4			
601.18	PKYVKLNTLKLAT	HA 307-319 analog	2.4		18.2		70.3	2083.3		14.7			71.8			
601.19	PKYVKTNTLKLAT	HA 307-319 analog	11.8		83.8		104.7	16666.7		47.1			122.8			
601.20	PKYVKQDTLKLAT	HA 307-319 analog	5.4		53.8		54.2	25000.0		80.5			681.4			
601.21	PKYVKQTTLKLAT	HA 307-319 analog	2.1		18.2		32.1	10000.0		40.8			373.1			
601.22	PKYVKQLTLKLAT	HA 307-319 analog	2.1		116.3		102.3	2000.0		73.5			332.1			
601.23	PKYVKQNSLKLAT	HA 307-319 analog	2.6		24.4		90.9	500000.0		1176.5			180.3			
601.25	PKYVKQNALKLAT	HA 307-319 analog	3.3		82.5		90.0	25000.0		150.4			180.3			
601.26	PKYVKQNTKKLAT	HA 307-319 analog	4.3		52.5		204.5	16666.7		512.8			26.0			
601.28	PKYVKQNTDKLAT	HA 307-319 analog	2.4		82.5		115.4	500000.0		6000.0			423.7			
601.29	PKYVKQNTLRLAT	HA 307-319 analog	1.1		24.4		62.5	300000.0		23.5			42.5			
601.30	PKYVKQNTLELAT	HA 307-319 analog	22.7		232.6		71.4	300000.0		645.2			240.4			
601.31	PKYVKQNTLSLAT	HA 307-319 analog	6.9		182.5		107.1	600000.0		10000.0			218.3			
601.32	PKYVKQNTLKQAT	HA 307-319 analog	6.2		122.0		30.0	50000.0		73.5			18.7			
601.33	PKYVKQNTLKKAT	HA 307-319 analog	1666.7		4.9		606.1	25000.0		392.3			510.2			
601.34	PKYVKQNTLKDAT	HA 307-319 analog	555.6		322.0		23.7	1562.5		53.8			842.1			
601.35	PKYVKQNTLKLST	HA 307-319 analog	3.6		30.5		47.9	25000.0		34.0			122.5			
601.36	PKYVKQNTLKLFT	HA 307-319 analog	2.8		21.2		40.0	25000.0		166.3			2500.0			
601.37	PKYVKQNTLKLKT	HA 307-319 analog	4.2		25.7		34.8	500000.0		53.6			138.9			
601.38	PKYVKQNTLKLAS	HA 307-319 analog	5.1		58.7		32.1	25000.0		47.1			83.3			
601.39	PKYVKQNTLKLAK	HA 307-319 analog	3.6		66.7		80.4	50000.0		118.0			104.2			
601.40	PKYVKQNTLKLAY	HA 307-319 analog	2.8		83.3		83.3	16666.7		107.8			47.3			
605.02	IQVYSRHPAENGKSNF	hu β2-μglob. 7-22	277.8		485.1	100000.0	155.2	50000.0		4000.0			2272.7			

Table VIII, page 6

In IC50 Format

Peptide	Sequence	Source	DR1 nM	DR2w201 nM	DR2w202 nM	DR3 nM	DR4w4 nM	DR4w14 nM	DR4w15 nM	DR5w11 nM	DR5w12 nM	DR6w19 nM	DR7 nM	DR8w2 nM	DR9 nM	DRw53 nM
606.02	BONVEDQTKGNR	Ova 143-158	553.8		(10000.0)	3000000.0	375.0	16666.7		200000.0			14.7			
611.01	PKYVKQNTLKLAT	HA 307-319 analog	2.8		27.1		30.0	50000.0		156.3			173.6			
611.02	PKYVKQNTLKLAA	HA 307-319 analog	30.5		40.7		60.0	25000.0		144.9			113.6			
611.03	PKYVKQNTLKAAT	HA 307-319 analog	10.2		48.8		5.0	357.1		61.9			1136.4			
611.04	PKYVKQNTLALAT	HA 307-319 analog	5.3		350.0		37.5	25000.0		1818.2			290.7			
611.05	PKYVKQNTAKLAT	HA 307-319 analog	6.8		44.3		84.3	500000.0		3333.3			510.2			
611.06	PKYVKQNALKLAT	HA 307-319 analog	1.7		14.6		882.4	600000.0		232.6			46.3			
611.07	PKYVKQATLKLAT	HA 307-319 analog	2.0		27.1		28.1	25000.0		111.1			337.8			
611.08	PKYVKANTLKLAT	HA 307-319 analog	2.9		16.2		46.4	50000.0		107.0			15.8			
611.09	PKYVAQNTLKLAT	HA 307-319 analog	3.9		48.8		14.1	16666.7		144.9			480.8			
611.10	PKYAKQNTLKLAT	HA 307-319 analog	5.7		1176.5		67.2	500000.0		800.0			1785.7			
611.12	PAYVKQNTLKLAT	HA 307-319 analog	2.9		18.2		23.7	50000.0		111.1			40.8			
611.13	AKYVKQNTLKLAT	HA 307-319 analog	4.5		18.2		19.6	25000.0		38.4			41.7			
611.14	PKYVKQNTLKLAI	HA 307-319 analog	4.6		80.0		84.3	50000.0		370.4			250.0			
611.21	PKYVKqNTLKLAT	HA 307-319 analog	35.7		117.8		47.4	500000.0		200000.0			2272.7			
611.29	pKYVKQNTLKLAT	HA 307-319 analog	3.6		50.0		45.8	25000.0		384.6			287.4			
611.30	PKYVKQNTLMLAT	HA 307-319 analog	8.8		250.0		61.1	7142.9		200.0			229.4			
611.31	PKYVKQNTKKLAT	HA 307-319 analog	8.1		36.5		321.4	600000.0		740.7			(6000.0)			
613.03	FRKNVTPRFPRKNVIPR	MBP reiterative	200.0		166.1	75000.0	4500.0	2000.0		1666.7			271.7			
640.04	ARRLKAIYARRLK	mouse reiterative	3.6		6.7	3000000.0	(37500.0)	666.7		666.7			29.2			
650.01	QYIKANSKFIGITE	Tet. Tox. 830-843	29.8		28.6	4166.7	(15000.0)	25000.0		21.1			11.4			
650.02	TYIKANSKFIGITE	Tet. Tox. 830-843	200.0		30.0	10000.0	(15000.0)	-50000.0		23.5			80.2			
650.03	LYIKANSKFIGITE	Tet. Tox. 830-843	170.8		153.6	100000.0	(15000.0)			117.6			131.6			
650.04	MYIKANSKFIGITE	Tet. Tox. 830-843	58.1		18.2	7894.7	(11250.0)	-50000.0		58.8			71.4			
650.07	QFIKANSKFIGITE	Tet. Tox. 830-843	10.2		11.1	4166.7	(11250.0)	-50000.0		57.1			6.9			
650.08	QYQKANSKFIGITE	Tet. Tox. 830-843	80.8		31.7	3000000.0	(11250.0)			2222.2			7.4			
650.09	QYKKANSKFIGITE	Tet. Tox. 830-843	24.8		125.0	300000.0	(11250.0)			2222.2			10.4			
650.10	QYIKANSKFIGITE	Tet. Tox. 830-843	32.1		30.2	37500.0	(15000.0)			21.1			6.1			
650.11	QYISANSKFIGITE	Tet. Tox. 830-843	34.7		90.9	21428.6	(11250.0)	-50000.0		88.7			17.9			
650.12	QYIAANSKFIGITE	Tet. Tox. 830-843	18.6		47.6	1587.3	(11250.0)	-50000.0		67.8			208.3			
650.15	QYIKFNSKFIGITE	Tet. Tox. 830-843	294.1		(5000.0)	3000000.0	(15000.0)			35.1			47.2			
650.17	QYIKAASKFIGITE	Tet. Tox. 830-843	43.9		22.3	1666.7	(15000.0)			8.0			17.5			
650.16	QYIKALSKFIGITE	Tet. Tox. 830-843	132.3		57.1	15789.5	6000.0	214.1		54.1			48.1			
650.19	QYIKADSKFIGITE	Tet. Tox. 830-843	68.4		57.1	100000.0	(6000.0)			105.3			33.3			
650.20	QYIKANYKFIGITE	Tet. Tox. 830-843	625.0		36.4	60000.0	(11250.0)			90.8			675.7			
650.22	QYIKANAKFIGITE	Tet. Tox. 830-843	9.1		27.8	5000.0	(11250.0)			83.3		3.5	22.7			
650.23	QYIKANSSFIGITE	Tet. Tox. 830-843	6.7		666.7	17647.1	12.5			117.6			177.3			
650.24	QYIKANSEFIGITE	Tet. Tox. 830-843	96.2		(1176.5)	300000.0	428.6			200000.0			271.7			
650.25	QYIKANSRFIGITE	Tet. Tox. 830-843	11.3		66.7	37500.0	7500.0			8.3			8.6			
650.26	QYIKANSKEGITE	Tet. Tox. 830-843	108.4		478.2	1255.2	6000.0	-50000.0		833.3			22.7			
650.27	QYIKANSKSIGITE	Tet. Tox. 830-843	10.8		111.1	1714.3	5000.0			100.0			16.7			
650.28	QYIKANSKYIGITE	Tet. Tox. 830-843	21.0		16.7	16666.7	7500.0			32.8			25.0			
650.30	QYIKANSKFQGITE	Tet. Tox. 830-843	2560.0		13.3	5555.6	937.5			33.6			625.0			
650.31	QYIKANSKFVGITE	Tet. Tox. 830-843	12.6		25.0	2727.3	(7500.0)			52.6			106.7			
650.32	QYIKANSKFIGITE	Tet. Tox. 830-843	70.4		117.0	75000.0	7500.0			571.4			757.6			
650.33	QYIKANSKFIYITE	Tet. Tox. 830-843	12.3		74.1	8333.3	(7500.0)			200000.0			147.1			
650.34	QYIKANSKFIAITE	Tet. Tox. 830-843	8.7		57.1	27272.7	(7500.0)			200.0			96.2			
650.35	QYIKANSKFIGQTE	Tet. Tox. 830-843	16.6		10.6	2564.1	(7500.0)			11.8			53.2			
650.36	QYIKANSKFIGKTE	Tet. Tox. 830-843	10.4		9.1	2459.0	(7500.0)	25000.0		5.3			33.3			
650.37	QYIKANSKFIGLTE	Tet. Tox. 830-843	27.8		18.7	6383.0	(7500.0)			21.7			92.8			
650.38	QYIKANSKFIGIKE	Tet. Tox. 830-843	47.2		20.0	8333.3	(15000.0)			14.3			27.2			
650.39	QYIKANSKFIGIFE	Tet. Tox. 830-843	64.8		16.7	6064.7	(7500.0)	-50000.0		38.5			520.8			
650.40	QYIKANSKFIGISE	Tet. Tox. 830-843	90.8		27.8	6172.4	(7500.0)			85.2			189.7			
650.41	QYIKANSKFIGITK	Tet. Tox. 830-843	11.0		13.3	15789.5	(7500.0)			60.6			58.1			
650.42	QYIKANSKFIGITD	Tet. Tox. 830-843	20.6		20.8	2903.7	(15000.0)			20.0			17.9			
650.43	QYIKANSKFIGITV	Tet. Tox. 830-843	48.1		21.3	14285.7	(15000.0)			57.1			37.9			
702.08	Ac-AAYAAAAAAKAAA-	Poly A DR binder	19.2		47.6		71.4	3333.3		1111.1			373.1			
702.10	Ac-aayaaaaakaaa	Poly A DR binder	29.3		1818.2		115.4	500000.0		714.3			1136.4			
703.03	YHIANEHPYCPHAMSALA	Ova 20 36	147.1		(10000.0)	42857.1	83.3	333.3		1111.1			(6250.0)			

Table VIII, page 7

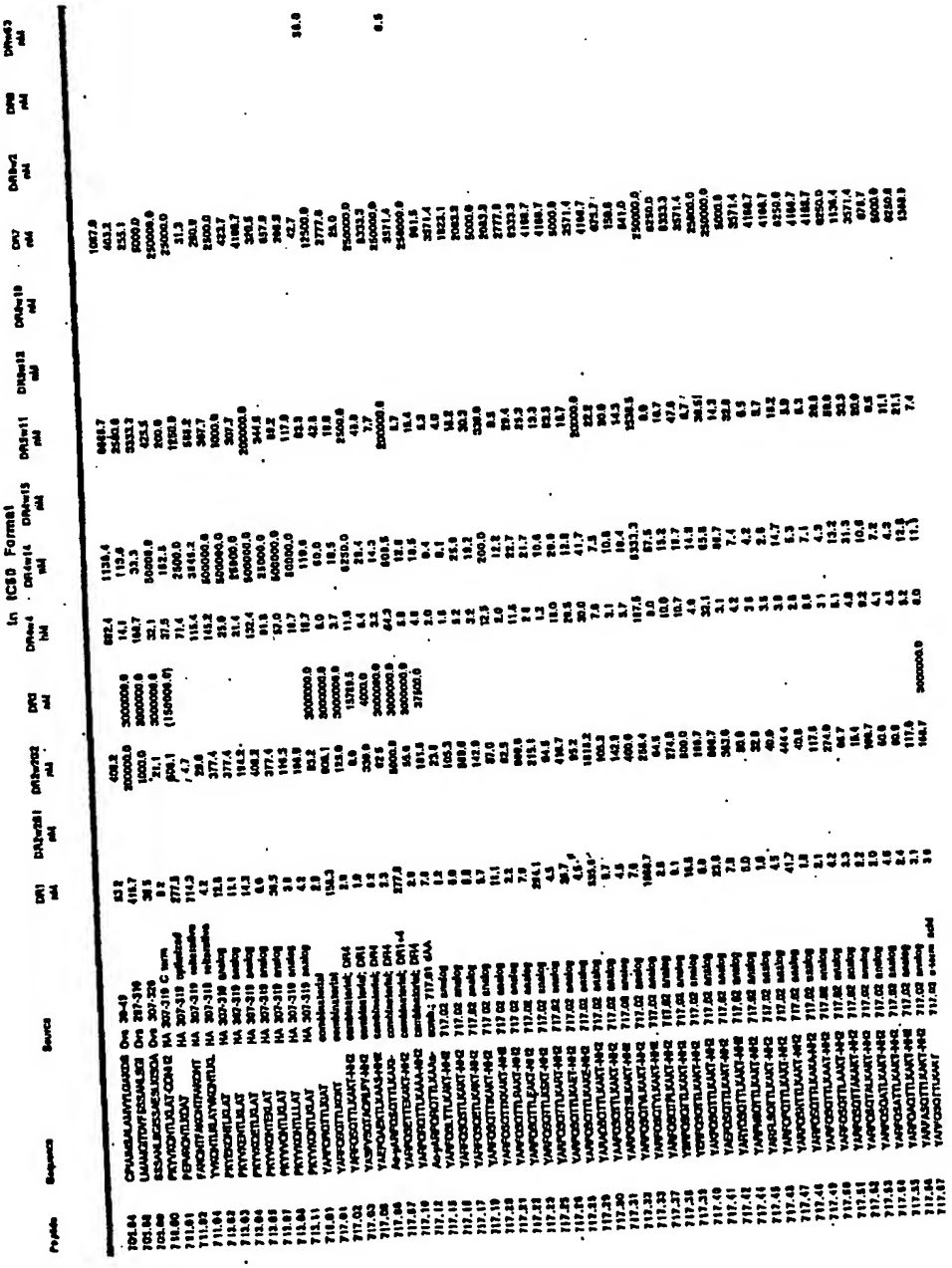

Table VIII, page 8

In IC50 Format

Peptide	Sequence	Source	DR1 nM	DR2w2β1 nM	DR2w2β2 nM	DR3 nM	DR4w4 nM	DR4w14 nM	DR4w15 nM	DR5w11 nM	DR5w12 nM	DR6w19 nM	DR7 nM	DR8w2 nM	DR9 nM	DRw53 nM
717.59	YQFVRANARSIQTTK	Tet Tox DR7	2.4		71.4	1036.1	88.3	78.4		8.0			13.2			
717.60	YAAYVIGLNAIRSS	HA DR7 combinatorial	13.8		80.0	3000000.0	1153.8	4166.7		125.0			373.1			
717.62	Ac-YARFQRQTTLKAAA-	717.10 modification	1.2		54.1	3000000.0	2.2	8.7		1.4			12500.0			
728.01	YEFVAAKAAQK	Tyr-1 HEL	15.7		74.1	3000000.0	692.3	500000.0		1818.2			1562.5			
723.01	YIAFLKQATAKLAT	Cytochrome	4.8		69.0	(150000.0)	16.7	746.3		30.5			15.8			
732.01	INSWVESQTHGIRNMLQPS	DQ pept. from Ova 145-	364.0		1333.3	(100000.0)	168.1	289.0		6666.7			266.0			
733.01	GVEQSSTRSSLYQLENYS	DQ pept. from human	277.8		200000.0	(100000.0)	107.1	74.8		370.4			1388.9			
734.02	LSQSHLVEALYLVSGERGFF	DQ pept. from human	14.4		2857.1	30000.0	865.4	1020.4		10000.0			123.8			
734.03	LVEALYLVSGERGFFYTPKT	DQ pept. from human	30.7		1818.2	1570.7	1153.8	704.2		2500.0			825.0			
735.01	KSNTATSATQRLANSLVH	DQ pept. from amylin 1-	312.5		200000.0	3000000.0	441.2	352.1		1333.3			12500.0			
735.02	TSATQRLANSLVHSSNNFG	DQ pept. from amylin 6-	111.1		10000.0	33333.3	5.4	13.2		129.0			373.1			
735.03	RLANSLVHSSNNFGAILSS	DQ pept. from amylin	84.7		10000.0	25000.0	11.8	12.0		202.0			555.6			
735.04	LVHSSNNFGAILSSTNVGS	DQ pept. from amylin	161.3		5000.0	15789.5	11.0	12.5		1250.0			961.5			
735.05	sNNFGAILSSTNVg-NH2	d-protected 735.04	46.5		2857.1	3000000.0	272.7	375.0		20000.0			12500.0			
738.08	AAL AAAAAAKAAA-NH2	Poly A descendent of	1.9		86.7		69.2	151.5		2222.2			1318.8			
738.12	JJY AAAAAAKAAA-NH2	Poly A descendent of	2.4		108.3		257.1	500000.0		1333.3			1785.7			
752.01	AVLEDPYILLVSSKV-NH2	MT 211-225	23.8	30.4	2857.1	75000.0	9000.0	333.3		6666.7			436.0			
758.01	QFVIANASSVAKTD	Tet Tox DR1	8.1		38.8	33333.3	28.1	45.5		444.4			367.0			
760.07	afaaakaaaafaay-NH2	retro-inverse poly A	50.0		1818.2		145.2	5555.6		1000.0			12500.0			
761.01	AAFAAAAQRLFA-NH2	poly A comb. control for	2.3		1.8	9375.0	30.0	8333.3		855.6			7.6			
762.01	aAYAAAAAAKAAa-NH2	N and C stabilized DR1	1.7		27.4		7.1	-12500.0		48.8			1666.7			
762.02	aAYAAAATAKAAa-NH2	N and C stabilized DR1	1.7		105.3	3000000.0	2.0	1428.6		25.6			684.4			
765.011	ASQKRPSQRSKYLATAST	Mouse MBP 1-20	312.5		2500.0	3000000.0	321.4	3031.8		4444.4			661.5			
765.02	KYLATASTMDHARHGFLP	Mouse MBP 11-30/	50000.0		10000.0	(100000.0)	100.0	88.5		5000.0			157.2			
765.09	NPVVHFFKNIVTPRTPPPS	Mouse MBP 81-100/	13.5		158.3	50000.0	7.4	35.7		16.7			66.8			
770.01	AFAAAATLKA-NH2	Truncated poly A	78.1		43.1	3000000.0	23.7	357.1		250.0			884.4			
770.02	Ac-AFAAAATLKA-NH2	Truncated poly A	35.7		132.5	3000000.0	21.4	102.0		103.6			324.7			
774.06	DALESIMTTKSVSFR	Rabies virus	364.8		20000.0	33333.3	109.8	60.0		8000.0			84.1			
778.00	LLVIVELIPSTSS	Ach Rec 257-269	67.8		2222.2	60000.0	28.1	62.5		1111.1			5000.0			
781.06	FQRQTTLKAAa-NH2	Truncated combinatorial	19.2		(2000.0)		2.4	13.9		(2222.2)			250000.0			
781.08	yARFQRQTTLKAAa-NH2	Truncated combinatorial	2.8		37.7		2.0	7.4		7.4			250000.0			
781.13	yArFQRQTTLKAAa-	Stabilized combinatorial	33.3		500.0	3000000.0	3.1	38.5		11.8			250000.0			
781.14	yAfFQRQTTLKAAa-	Stabilized combinatorial	16.1		153.8	3000000.0	8.1	28.3		6.0			250000.0			
781.16	yAAFQRQTTLKAAa-NH2	Stabilized combinatorial	3.0		153.8	3000000.0	3.8	7.0		16.0			2777.8			
781.19	yArFQKQTTLKAAa	Stabilized combinatorial	66.2		3333.3	3000000.0	8.1	66.6		47.5			260000.0			
782.02	YARFASQTTLKAKT-NH2	717.02 modification	3.0		190.5	3000000.0	2.5	14.7		25.7			6250.0			
783.01	GAAYAAAAAAKAAA-	*Acetylation effect	4.2		16.6	(150000.0)	37.5	500000.0		118.3			543.5			
792.01	PKYVKQNTLKLAT	HA 307-319 analog	30.0		3333.3		173.1	294.1		10000.0			250000.0			
792.02	PKYVKQNTLKFAT	HA 307-319 analog	108.7		27.4		86.2	500000.0		20000.0			1087.0			
799.01	PLLVLQAGFFLLTRILTIPQ	HBs (ayw) 11-30	172.4		4000.0	3000000.0	241.8	80.3		41.3			89.2			
800.01	MQQNLSTSNPLGFFPDH	Pre S (1) ayw 1-17	94.3		1538.5	6818.2	66.8	2000.0		2000.0			154.3			
801.02	NPVLTTASPLSSIFSRI	Pre S (2) ayw 33-49	714.3		20000.0	300000.0	54.1	8.6		200000.0			63.1			
802.02	CNQFLMTLATWVGNNLED	Core F 81-90	357.1		250000.0	100000.0	737.7	80.7		6000.0			6250.0			
802.04	GRETVIEYLVSFGVW	Core H 111-125	12.8		1333.3	16666.7	833.3	32.3		1538.5			87.8			
803.06	aFAAAKTAAAAa-CONH2	760.15 truncation	8.8		303.0	300000.0	1.0	41.7		36.4			6250.0			
803.07	aAFAAAKTAAAAa-	760.15 truncation	6.8		41.2	100000.0	1.3	18.2		5.3			306.8			
806.02	Ac-yQRIKANSKFIGIKFa-	DR2 combinatorial	277.8		1.6		361.4	8333.3		6.3			543.5			
806.03	YAAYVKLTAARKFA-NH2	DR2 combinatorial	2.1		0.7	23076.9	21.4	4545.5		1.3			4.4			
806.04	Ac-yAAYVKLTAARKFa-	DR2 combinatorial	4.2		1.1		80.8	3571.4		8.8			14.7			
812.01	QYIKANSKFIGITELKK-NH2	Tet Tox 830-846	80.6		14.0		408.1	16666.7		28.6			357.1			
812.02	ILMQYIKANSKFIGITE	Tet Tox 827-843	13.9		2.6		37.5	131.6		7.7			7.1			
812.03	LMQYIKANSKFIGITE	Tet Tox 828-843	13.2		4.8		150.0	85.8		13.9			11.8			
812.04	QYIKANSKFIGITE	Tet Tox 830-843	60.2		10.3		1551.7	25000.0		8.3			13.8			
812.05	MQYIKANSKFIGITE	Tet Tox 829-843	64.1		2.4		229.0	84.8		8.7			7.8			
818.01	PKFVKQNTLKLAG	HA 307-319 analog	4.3		36.7		45.8	357.1		125.0			174.0			
818.02	PKFVKQNTLKLGT	HA 307-319 analog	14.8		208.3		112.6	3571.4		888.8			185.2			
818.03	PKFVKQNTLKGAT	HA 307-319 analog	34.5		173.4		7.3	35.7		18.2			2500.0			
818.04	PKFVKQNTLGLAT	HA 307-319 analog	8.5		95.2		30.1	543.5		181.8			403.2			
818.05	PKFVKQNTGKLAT	HA 307-319 analog	19.8		46.5		230.8	686.7		1179.6			8333.3			

Table VIII, page 9

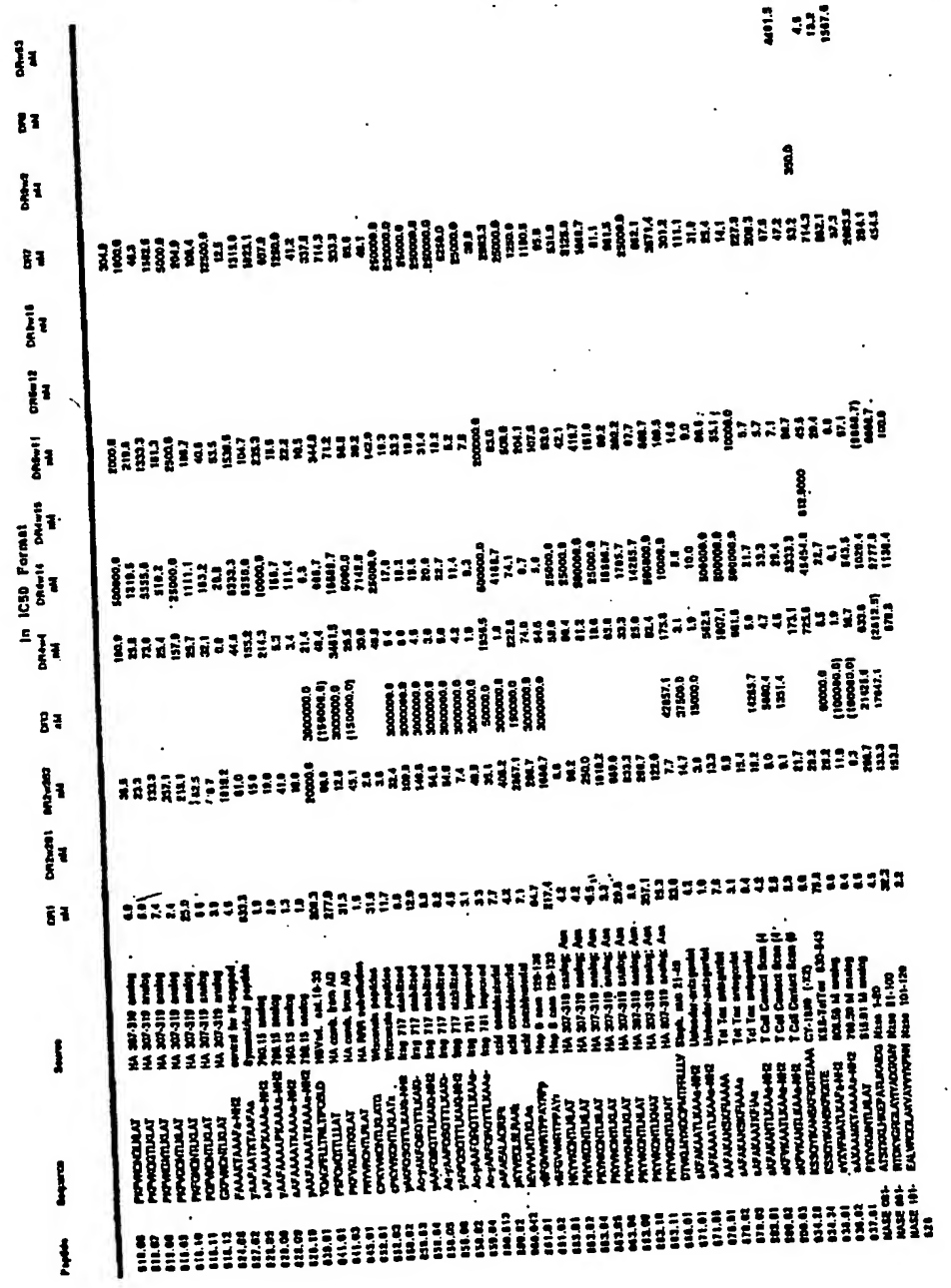

TABLE IX

MOTIFS		POSITION 1° anchor 1	2	3	4	5	1° anchor 6	7	8	9
DR4	preferred	FMY*LIVW*	M	T		I	VST*CPALIM*	MH		MH
	deleterious				W			R		WDE
DR1	preferred	MF*LIVWY*			PAMQ		VMAT*SPLIC*	M		AVM
	deleterious		C	CH	FD	CWD		GDE	D	
DR7	preferred	MF*LIVWY*	M	W	A		IVMSA*CTPL*	M		IV
	deleterious		C		G			GRD	N	G
DR Supermotif		MF*LIVWY*					VMSTA*CPLI*			

DR3 MOTIFS	1° anchor 1	2	3	1° anchor 4	5	1° anchor 6
motif a preferred	LIVMFY			D		
motif b preferred	LIVMFAY			DNQEST		KRH

Italicized residues indicate less preferred or "tolerated" residues.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a unit dose form of a peptide comprising an epitope from Table VIII or analog thereof which binds to an HLA class II molecule at an IC_{50} of less than or equal to 1,000 nM.

2. The composition of claim 1, wherein the peptide is derived from a tumor antigen which is selected from the group consisting of carcinoembryonic antigen (CEA), p53, MAGE-2, MAGE-3, or Her2/neu.

3. The composition of claim 1, wherein the immunogenic peptide is derived from a viral antigen.

4. The composition of claim 3, wherein the viral antigen is from HIV, HBV, or HCV.

5. The composition of claim 5, wherein the antigen is *Plasmodium falciparum.*

6. A composition of claim 1 wherein the epitope comprises an amino acid that is Y, F, W, L, I, V, or M at the first position from the N-terminus of the epitope and an amino acid of S, T, C, A, P, V, I, L, or M at the sixth position from the N-terminus of the epitope.

7. A composition of claim 1 wherein the composition is a nucleic acid that encodes the peptide.

8. A method of inducing a helper T cell response in a patient, the method comprising contacting a helper T cell with a composition of claim 1.

9. The method of claim 9, wherein the composition is a nucleic acid that encodes the peptide.

10. A composition comprising an epitope from Table VIII or analog thereof which binds to an HLA class II molecule at an IC_{50} of less than or equal to 1,000 nM wherein the epitope is bound to an HLA class II molecule present on an antigen presenting cell.

11. A composition that comprises at least two peptides of claim 1.

12. A composition that comprises at least three peptides of claim 1.

13. A composition of claim 1, wherein a unit dose form of the peptide is in the range of between 500 μg and 50,000 μg.

RESIDUE	p1 ANCHOR	2	3	4	5	p6 ANCHOR	7	8	9
C		0.57	0.74	1.12	0.83	0.47	0.94	0.28	1.10
G		1.14	0.64	0.43	0.48		0.49	1.19	0.52
S		1.55	1.31	1.29	1.76	1.11	1.23	2.93	1.54
T		1.00	4.34	0.89	1.32	1.86	3.07	1.76	1.64
P		0.56	0.31	1.44	2.46	0.86	2.83	2.12	2.18
A		0.96	1.04	1.57	0.59	0.65	0.86	0.82	1.62
L	0.81	0.86	1.88	1.28	1.11	0.67	1.36	1.08	0.83
I	0.79	1.74	1.01	1.91	4.39	0.98	2.36	1.66	2.75
V	0.79	3.34	0.93	1.05	0.70	2.36	0.69	0.54	1.53
M	1.14	12.79	1.49	2.77	0.32	0.74	8.11	1.98	4.05
F	2.33	3.66	1.85	0.80	1.58		1.84	1.34	1.12
W	0.82	2.04	2.52	0.21	0.91		0.39	0.35	0.22
Y	1.07	0.74	1.51	0.39	1.41		0.44	0.61	0.35
H		0.78	0.15	1.14	0.93		13.77	1.40	5.15
R		1.09	0.50	0.69	0.39		0.14	0.41	1.22
K		1.44	1.25	0.53	0.40		0.62	0.64	0.55
Q		0.40	0.38	1.61	2.09		0.31	0.71	0.62
N		0.44	1.72	1.42	1.89		0.84	0.43	1.64
D		0.34	0.33	1.40	0.40		0.58	0.53	0.24
E		0.31	1.09	0.42	0.42		0.29	0.61	0.25

FIG. 1.

RESIDUE	p1 ANCHOR	2	3	4	5	p6 ANCHOR	7	8	9
C		0.22	0.15	0.49	0.06	0.14	0.31	0.45	0.35
G		1.29	3.38	2.13	1.73		0.23	1.58	0.44
S		0.87	0.48	0.32	0.58	0.74	1.03	1.25	1.03
T		0.57	2.08	0.30	1.59	1.26	1.51	1.73	2.32
P		0.43	0.88	5.42	2.57	0.63	1.78	1.63	1.52
A		1.93	3.51	4.14	1.59	2.42	1.89	1.25	4.09
L	0.97	1.20	0.64	3.08	2.32	0.85	2.02	3.10	0.83
I	1.00	3.84	1.59	1.10	1.30	0.75	3.47	0.67	1.32
V	0.74	2.95	1.08	0.79	1.97	1.16	2.89	0.57	5.89
M	2.82	1.07	2.62	7.66	0.93	2.67	7.27	1.01	4.39
F	1.51	2.05	0.49	0.22	0.40		0.91	0.89	0.79
W	0.30	0.63	0.69	0.56	0.14		0.61	0.35	0.58
Y	0.88	0.51	1.22	0.36	2.04		0.99	0.26	0.42
H		0.51	0.11	0.68	1.57		1.81	1.20	0.55
R		0.80	0.49	0.43	0.37		1.08	1.43	0.83
K		2.69	2.32	0.49	0.67		1.33	2.24	0.44
Q		1.38	1.27	7.07	1.58		1.06	3.65	1.54
N		0.63	1.41	1.20	0.75		1.16	0.43	1.15
D		0.85	0.31	0.20	0.21		0.11	0.08	0.39
E		0.31	0.47	0.59	0.57		0.16	0.53	0.27

FIG. 2.

RESIDUE	p1 ANCHOR	2	3	4	5	p6 ANCHOR	7	8	9
C		0.17	0.58	0.30	0.26	0.45	1.38	0.53	1.04
G		0.45	0.43	0.25	0.54		0.23	1.30	0.22
S		1.86	0.66	1.11	2.39	1.14	1.95	1.67	0.89
T		0.72	6.53	1.88	1.78	0.79	1.54	0.94	1.92
P		0.36	0.37	2.01	0.46	0.49	1.06	0.60	1.78
A		1.43	2.63	4.78	0.89	1.51	0.74	0.89	0.61
L	0.87	1.04	1.08	1.09	0.83	0.89	1.88	1.18	0.97
I	0.77	1.99	0.96	2.17	2.88	1.11	1.11	1.52	5.69
V	0.82	2.15	0.47	0.57	0.92	2.25	1.36	0.80	5.49
M	1.45	5.75	2.54	3.74	0.33	1.21	9.03	3.01	3.42
F	1.97	1.43	0.68	0.90	1.07		2.50	2.39	1.90
W	0.93	1.32	4.07	0.81	0.58		0.81	0.95	0.66
Y	0.90	0.78	3.34	0.62	3.32		0.64	0.74	0.74
H		1.67	0.36	0.62	2.09		1.10	1.02	1.13
R		1.29	0.70	0.45	1.31		0.21	0.59	2.67
K		1.45	1.32	0.47	0.86		1.40	1.26	0.48
Q		1.70	0.82	2.09	1.4		1.01	2.68	0.36
N		1.42	2.35	0.86	1.68		1.62	0.24	0.88
D		0.61	0.41	0.27	0.26		0.19	0.44	0.30
E		0.48	0.59	1.23	0.74		0.45	0.57	1.16

FIG. 3.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/12066

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.23, 7.24, 343.2, 344; 424/160.1, 159.1, 174.1, 188.1, 189.1, 208.1, 227.1; 530/388.35, 388.3, 388.75, 388.8, 389.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG, BIOSIS, DISSERTATION ABSTRACTS ONLINE, EMBASE, MEDLINE, AIDSLINE,epitope, carcinoembryonic antigen, HLA, MAGE, HIV, CLASS II, tumor antigen

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	VALMORI ET AL. Analysis of MAGE-3-specific Cytolytic T Lymphocytes in Human Leukocyte Antigen-A2 Melanoma Patients. Cancer Research. 15 February 1997. Vol 57. No. 4. pages 735-741, especially Abstract.	1-13
Y	HARRISON ET AL. A Peptide-binding Motif for I-Ag7, the Class II Major Histocompatibility Complex (MHC) Molecule of NOD and Biozzi AB/H Mice. J. Exp. Med. 17 March 1997. Vol 185. No. 6. pages 1013-1021, especially Abstract.	1-13

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"B" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 AUGUST 1999	21 OCT 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer HANNEL [illegible], PH.D.
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/12066

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FRAZIANO ET AL. The Presence of Antibodies against HIV Peptides in the Sera of Alloimmune Mice and Thalassemic Patients Is Due to a Polyclonal Activation mechanism. Clinical Immunology and Immunopathology. August 1997. Vol 84. No. 2. pages 202-207, especially Abstract.	1-13
Y	BREMERS, ET AL. The Use of Epstein-Barr Virus-Transformed B Lymphocyte Cell Lines in a Peptide-Reconstitution Assay: Identification of CEA-Related HLA-A *0301-Restricted potential Cytotoxic T-Lymphocyte Epitopes. J. Immunotherapy. August 1995. Vol 18. No. 2. pages 77-85, especially Abstract.	1-13
Y	ZAREMBA ET AL. Identification of an Enhancer Agonist Cytotoxic T lymphocyte Peptide from Human Carcinoembryonic Antigen. Cancer Research. 15 October 1997. Vol 57. pages 4570-4577, especially Abstract.	1-13
Y	RAS ET AL. Identification of Potential HLA-A *0201 Restricted CTL Epitopes Derived from the Epithelial Cell Adhesion Moleucle (Ep-CAM) and the Carcinoembryonic Antigen (CEA). Human Immunology. January 1997. Vol 53. pages 81-89, especially Abstract.	1-13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/12066

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

G01N 33/574, 33/53; C12N 7/00; A61K 39/42, 39/395, 39/21, 39/29; C07K 16/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/7.23, 7.24, 343.2, 344; 424/160.1, 159.1, 174.1, 188.1, 189.1, 208.1, 227.1; 530/388.35, 388.3, 388.75, 388.8, 389.4